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INDUCED MUTAGENESIS USING GAMMA RADIATIONS IN *Tagetes erecta*

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ABSTRACT

Marigold is a popular loose flower crop having good market. So, there is always demand of novel varieties in marigold. Induced mutagenesis is a very convenient and fast method to create new variations. By using mutagens, mutations were induced by treating seeds or any propagating material. In this investigation, we treated marigold seeds with gamma radiations and observed that the parameters i.e. plant length, plant spread, number of branches, flower size, ray floret length and width were significantly decreased with increase in doses of gamma rays. At lower doses, the growth was significantly good but later it decreased and had deleterious effect at higher doses to marigold. The days to bud initiation and full bloom and flower duration increased with increase in doses of gamma rays. Induced mutations resulted in changes in visual parameters with respect to plant stature and flower structure. Flower colour changes from shades of yellow orange to yellow from orange that is original colour of variety "Punjab Gainda No.1".

Keywords: Gamma rays, Induced Mutagenesis, Marigold, Mutations, Variation.

Marigold belongs to the family *Asteraceae* and is native to Central and South America. It is used as a loose flower crop commercially, as pot plant and also as bedding or edging plant in landscaping. Marigold is very adaptive to different habitats; it thrives best in hot and dry as well as humid weather conditions, under both tropical and subtropical conditions. It is propagated through seeds commercially and with terminal cuttings for clonal propagation. Many workers have tried to improve marigold crop by different breeding methods resulting in development of new cultivars. Among them, mutation is very popular and easy method specially, for ornamental plants. In ornamentals, we don't have to care about nutritional value as they are non-edible unlike of other agricultural crops where nutrition and taste is foremost approach to improve the crop. Ornamentals are for eye pleasing material, which should look good for garland making or as bedding plant in landscape. In ornamental plants, parameters deciding commercial aspects i.e. morphological or flower traits can be easily altered with induced mutations by application of artificial mutagens. Induced mutation is an important complementary method for the genetic improvement of many crop species and has great potential especially for floriculture industry. The utilization of induced mutations of crop plants has created several mutants which have been used directly as new cultivars. Many artificial mutagens like gamma rays, X-rays, EMS and NMU have been used for obtaining desirable mutants in various ornamental plants like chrysanthemum, rose and gladiolus etc. The gamma rays are high ionizing radiations which affect the living system inside seed by effecting its energy system and further causing damage to living system. The water molecules present in seeds are ionized into free radicals and thus biological system produces reactive oxygen species (ROS), as the result H^+ ions become trapped (Esnault *et al.,* 2010) and these free radicals can damage components of plant cells and further can affect biochemical and physiological processes occurring in plants. So, gamma rays are good tool for induction of mutations artificially for improvement of our ornamental crops.

MATERIALS AND METHODS

In the present investigations, seeds of African marigold cv. 'Punjab Gainda No.1' were treated with gamma rays (^{60}Co) with different doses i.e. 0 (control), 50, 100, 150, 200, 250 and 300 Gy at College Orchard, Department of Fruit Science, Punjab Agricultural University, Ludhiana using Gamma Rays Low Dose Irradiator 2000. Seeds treated were then sown as M_1 generation in February, 2018, and seed obtained from these plants were sown as M_2 generation in July, 2018 at Research farm, Department of Floriculture and Landscaping, PAU, Ludhiana. Seedling sown on raised bed was transplanted to field in randomized block design at (40 × 40) cm distance from plant to plant and row to row respectively. The various parameters observed were field survival percentage, plant height, plant spread, number of branches per plant, days to bud initiation, days to full bloom, flowering duration, number of flowers per plant, flower size, ray floret length, rays floret width, leaf abnormalities percentage, floral abnormalities percentage and visible changes in leaves and flower colour, form and shape.

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RESULTS AND DISCUSSION

Marigold is easily propagated by seeds. In this study, seeds were treated with gamma radiations to induce variations and studied their effect on growth and flowering parameters.

Persual of data regarding field survival percentage showed significant effect by treatment with gamma rays in marigold (Table 1). The maximum mean field survival percentage (39.85%) was in control better than all other treatments and minimum (24.26%) in 300 Gy, irrespective of mutation generations. Among mutation generations, significant results were obtained with maximum (32.22%) in M_1 generation followed by M_2 generation (29.93%), irrespective of different doses of gamma rays. In the data of interaction, results were significant between different doses of gamma rays and mutation generations. Survival percentage of marigold plants was reduced with increase in doses of gamma radiations. Ionizing radiations may cause reduction in hydrolysis of water in vital processes occurring in plant cell thus affecting survival rate of plants. The gamma rays may inactivate auxin synthesis and thus affect cell division and cell growth of plants. The lethal effect of harmful gamma rays cause damage to genetic material and may cause cell death of plants at higher doses of gamma rays. The present findings were in confirmation with Singh *et al.* (2009) who observed 100% survival in marigold control to 68.5% at 400 Gy. Banerji and Datta (1993, 2001, 2002) explained that survival of plants depends upon degree of damage caused to chromosomal material present in plant cells in chrysanthemum. Also, similar results were obtained in other crops, chrysanthemum and gladiolus.

The growth of plant reduced by treatment with gamma rays and showed significant effect on plant height, plant spread and number of branches per plant (Table 1). The plant height and plant spread decreased with increasing doses of gamma rays. The mean plant height was maximum (66.28 cm) in 50 Gy which was found to be at par with control (61.19 cm) and 100 Gy (58.78 cm) and was significantly better than all the other treatments. The plant height was found minimum (38.23 cm) in 300 Gy and was at par with 250 Gy (46.34 cm), irrespective of mutation generations. The mean plant spread was maximum in 50 Gy (52.63 cm) and was at par with treatment 100 Gy (48.22 cm) and 150 Gy (45.65 cm) and was significantly better than all the others treatments and minimum (26.55 cm) in 300 Gy, irrespective of mutation generations. Among mutation generations, non-significant results were observed with maximum plant height (54.68cm) in M_1 generation followed by M_2 generation (53.45 cm) and maximum plant spread (44.58cm) was in M_1 generation followed by M_2 generation (40.90 cm), irrespective of different doses of gamma rays. In the data of interaction between them were non-significant for both plant height and plant spread. At lower concentrations 50 Gy and 100 Gy, plant height was significantly at par with control, its mean lower doses were unable to affect plant height and may had stimulating effect at 50 Gy dose on plant height but at higher doses, it had deleterious effect on plant height. The gamma rays treatment degrades auxins and inactivates auxin synthesis and thus resulted in reduction in plant height of marigold. These results were in confirmation with the earlier findings of Singh *et al.* (2009), who observed plant height reduced from 62.09 cm (control) to 45.65 cm (300 Gy) and 40.32 cm (400 Gy) in marigold in cv. Pusa Narangi Gainda. Kole and Meher (2005) treated zinnia seeds with gamma rays and had found significant increase in plant height over control at lower doses followed by decrease in higher doses. Similarly, Banerji and Datta (2002) in chrysanthemum explained that reduction in plant height depends upon extent of nature and damage caused to chromosomes. Similar results were observed by Dilta *et al.* (2006) in *Dendranthema grandiflora* cv. 'Gulmohar' and Mishra *et al.* (2009) in chrysanthemum.

With increased doses of gamma rays, plant spread decreased. The results on plant spread are in conformity with earlier findings of Singh *et al.* (2009), they reported that in marigold, plant spread decreased from 29.24 cm (control) to 18.63 cm (300 Gy) and 17.59 cm (400 Gy) in North-South plant spread and from 28.35 cm (control) to 17.20 cm (400 Gy) in East- West plant spread. Dilta *et al.* (2003) irradiated rooted cuttings of chrysanthemum and decreased plant spread after gamma irradiation.

The mean number of branches per plant were maximum (5.62) in 50 Gy and were at par with control (5.20) and 100 Gy (5.03) and significantly better than all the other treatments, irrespective of mutation generations. The mean number of branches per plant were minimum in 300 Gy (4.25) and were at par with 150 Gy (4.83), 200 Gy (4.68), 250 Gy (4.55). Among mutation generations, results were significant and maximum (5.07) were recorded in M_1 generation followed by M_2 generation (4.69), irrespective of different doses of gamma rays. In the data of interaction, non-significant results were obtained. This indicates that the lower doses of gamma rays were at par with control on number of branches and then with increase in gamma rays dose, number of branches per plant decreased. At higher doses, growth of plant were affected due to chromosomal aberrations. These were in agreement with Singh *et al.* (2009), they reported that with increase in gamma radiations doses, number of branches reduced from 5.45 cm (Control) to 3.77 cm (400Gy) in marigold. Similar results were found by Datta and Banerji (1993) and Mishra *et al.* (2009) in chrysanthemum.

Table 1. Effect of gamma rays on field survival percentage, plant height, plant spread, number of branches per plant and leaf abnormalities percentage of marigold in different mutation generations

Treatments (Gy)	Field survival percentage (%)			Plant height (cm)			Plant spread (cm)			Number of branches per plant			Leaf abnormalities percentage		
	M_1	M_2	Mean	M_1	M_2	Mean	M_1	M_2	Mean	M_1	M_2	Mean	M_1	M_2	Mean
0 (Control)	50.07 (45.02)	32.40 (34.68)	41.23 (39.85)^{a}	61.63	60.750	61.19^{ab}	46.37	42.44	44.40^{bcd}	5.07	5.33	5.20^{ab}	0.00 (0.00)	0.00 (0.00)	0.00 (0.00)^{g}
50	37.33 (37.65)	28.27 (32.11)	32.80 (34.88)^{b}	69.23	63.333	66.28^{a}	55.25	50.00	52.63^{a}	5.73	5.50	5.62^{a}	4.10 (11.57)	5.44 (13.43)	4.77 (12.50)^{f}
100	28.40 (32.19)	28.40 (32.19)	28.40 (32.19)^{c}	60.23	57.333	58.78^{abc}	49.92	46.52	48.22^{ab}	5.25	4.80	5.03^{abc}	10.33 (18.68)	7.27 (15.62)	8.80 (17.15)^{e}
150	24.13 (29.41)	26.40 (30.90)	25.27 (30.16)^{d}	55.00	54.250	54.63^{bcd}	46.83	44.47	45.65^{abc}	5.00	4.67	4.83^{bcd}	15.98 (23.51)	16.16 (23.66)	16.07 (23.58)^{d}
200	22.13 (28.05)	23.00 (28..64)	22.57 (28.35)^{e}	53.23	52.750	52.99^{bcde}	45.00	42.50	43.75^{bcde}	5.08	4.27	4.68^{bcde}	20.24 (26.66)	20.58 (26.93)	20.41 (26.80)^{c}
250	21.33 (27.48)	22.40 (28.27)	21.87 (27.86)^{ef}	47.27	45.417	46.34^{def}	38.67	37.29	37.98^{cdef}	4.83	4.27	4.55^{bcdef}	31.46 (34.06)	28.57 (32.29)	30.02 (33.18)^{b}
300	18.87 (25.73)	15.00 (22.78)	16.93 (24.26)^{g}	36.13	40.333	38.23^{fg}	30.04	23.07	26.55^{fg}	4.50	4.00	4.25^{defg}	38.65 (38.42)	46.67 (43.07)	42.66 (40.74)^{a}
Mean	28.90 (32.22)^{a}	25.12 (29.93)^{b}		54.68^{a}	53.45^{a}		44.58^{a}	40.90^{a}		5.07^{a}	4.69^{b}		17.25 (21.84)^{a}	17.81 (22.14)^{a}	
CD (5%)	Mutation generations (A) = 0.36 ; Doses of gamma rays (B) = 0.68 ; Interaction (A×B) = 0.96			Mutation generations (A) = NS ; Doses of gamma rays (B) = 9.99 ; Interaction (A×B) = NS			Mutation generations (A) = NS; Doses of gamma rays (B) = 7.95; Interaction (A×B) = NS			Mutation generations (A) = 0.35; Doses of gamma rays (B) = 0.66 ; Interaction (A×B) = NS			Mutation generations (A) = NS ; Doses of gamma rays (B) = 2.15 ; Interaction (A×B) = 3.04		

* Figures in parentheses are arcsine values* Different letters in each column are significantly different at P≤0.05 by Duncan's Multiple Range Test (DMRT)

Flower characters like flower size, ray floret length, ray floret width and number of flowers per plant varied significantly with treatment with different doses of gamma rays in marigold (Table 2). The mean flower size was maximum (6.30cm) in 50 Gy was at par with 100 Gy (5.83cm) better than all other treatments and minimum (4.85 cm) in 300 Gy was at par with 250 Gy (5.31 cm), irrespective of mutation generations. Among mutation generations, results were non-significant but maximum flower size (5.67cm) was observed in M_1 generation followed by M_2 generation (5.49 cm), irrespective of different doses of gamma rays. The results of interaction had non-significant effect on flower size. The results indicated that with increase in doses of gamma rays, flowers size decreased. The flower size of marigold in this study was significantly same at lower doses and in control but highest in 50Gy dose of gamma rays and later it decreased with increase in doses of gamma rays. Sometimes, lower doses prove to have stimulatory effect on plants and thus increased some growth parameters by deactivating inhibitors present in plants which otherwise inhibit the plants from growth. These results were in confirmation with study of Singh *et al.* (2009) in marigold and by Kumari *et al.* (2013) in chrysanthemum

In the present study, the results indicates that with increase in doses of gamma rays, ray floret length and ray floret width decreased. The ray floret length and width increased significantly at lower doses and then reduced with increase in doses of gamma rays. The mean ray floret length was maximum (2.06 cm) in 50 Gy better than all other treatments and minimum (1.66 cm) in 300 Gy and was at par with 250 Gy (1.80 cm), irrespective of mutation generations. The mean ray floret width was maximum (1.56 cm) in 50 Gy and was at par with control (1.49 cm), 100 Gy (1.50 cm) and 150 Gy (1.46 cm) better than all other treatments and minimum (1.26 cm) in 300 Gy was at par with 200 Gy (1.36 cm) and 250 Gy (1.34 cm), irrespective of mutation generation. Among mutation generations, results were non-significant both for maximum ray floret and floret width and floret length (1.89 cm) was in M_1 generation followed by M_2 generation (1.84 cm) and maximum ray floret width (1.45 cm) was in M_2 generation followed by M_1 generation (1.39 cm), irrespective of different doses of gamma rays. Data of Interaction revealed between different doses of gamma rays and mutation generations were also non-significant for both ray floret length and width. The reduction in flower ray length and width with higher doses was due to damage caused to vegetative growth and disturbances occurred in physiological processes occurring in plant. Our results are in confirmation with Singh *et al* (2009), they reported that with decrease in ray floret length with increasing gamma rays doses from 2.69 cm (control) to 1.52 cm (400 Gy) with increasing dose of gamma rays and Datta and Tondon (1994) in chrysanthemum cultivars and similar results for ray floret width by Singh *et al.* (2009) and Datta and Tondon (1994) in chrysanthemum.

Similarly, results indicate that with increase in gamma rays doses, number of flowers per plant decreased. The mean number of flowers per plant was maximum (50.99) in 50 Gy and minimum (36.58) in 300 Gy, irrespective of mutation generations. Among mutation generations, results were significant and was maximum (44.75) in M_1 generation followed by M_2 generation (39.85), irrespective of different doses of gamma rays. In the data of interaction between different doses of gamma rays and mutation generations, results obtained were non-significant. The number of flower produced was not significantly affected by gamma radiations but there was reduction in production of flower with increased gamma rays doses. This reduction is due to decrease in growth parameters by gamma rays. Dilta *et al.* (2003) irradiated rooted cuttings of chrysanthemum and studied that number of flowers decreased after gamma irradiation treatment. Sisodia and Singh (2014) in gladiolus, Singh *et al.* (2009) in marigold and Mishra *et al.* (2009) in chrysanthemum got the same results.

The different doses of gamma rays to marigold seeds had significant effect on leaf abnormalities percentage (Table 1) and floral abnormalities percentage (Table 2). Leaf abnormalities includes change in size of leaves (smaller and larger leaves, narrow and broad leaves), assymetrical leaf shape, fused leaves, leathery texture of leaves in few cases, change of colour from dark green, green to pale green and also with chlorophyll and leaf variegation in fewer cases. These all abnormalities were due to change in genetic material and chromosomal abbreviations due to exposure to high doses of ionized radiations (gamma rays). The mean leaf abnormalities percentage was maximum (40.74 %) in 300 Gy significantly more than all other treatments and had no effect in control that is (0.00%) as they are totally normal plants, irrespective of mutation generations. Among mutation generations, results were non-significant and maximum leaf abnormalities percentage (22.14 %) was observed in M_2 generation followed by M_1 generation (21.84%), irrespective of different doses of gamma rays. The results of interaction, data was significant between different doses of gamma rays and mutation generations. These results indicate that with increase in doses of gamma rays, leaf abnormalities increased. There were number of abnormal leaves with exposure to gamma radiations. These leaf abnormalities gradual increased with increasing higher doses was in agreement with Singh *et al* (2009), they reported increase in leaf abnormalities from 0 (control) to 53.57% with 400 Gy in marigold cv. *Pusa Narangi Gainda*. Misra (1998) also reported deformed, narrow leaves and leaf variegation

Table 2. Effect of gamma rays on flower size (cm), ray floret length (cm), ray floret width (cm), number of flowers per plant and flower abnormalities percentage of marigold in different mutation generations

Treatments (Gy)	Flower size (cm)			Ray floret length (cm)			Ray floret width (cm)			Number of flowers per plant			Flower abnormalities percentage		
	M_1	M_2	Mean	M_1	M_2	Mean	M_1	M_2	Mean	M_1	M_2	Mean	M_1	M_2	Mean
0 (Control)	5.77	5.66	5.72^{bc}	1.93	1.84	1.88^{bc}	1.40	1.55	1.48^{abc}	47.60	40.42	44.01^{a}	0.00 (0.00)	0.00 (0.00)	0.000 (0.00)^{g}
50	6.29	6.31	6.30^{a}	2.04	2.08	2.06^{a}	1.52	1.61	1.56^{a}	55.40	46.58	50.99^{a}	6.95 (15.26)	14.18 (22.10)	10.57 (18.68)^{f}
100	5.85	5.81	5.83^{ab}	1.95	1.88	1.92^{ab}	1.47	1.53	1.50^{ab}	45.20	43.50	44.35^{a}	17.14 (24.40)	19.48 (26.17)	18.31 (25.28)^{e}
150	5.72	5.50	5.61^{bcd}	1.91	1.81	1.86^{bcd}	1.46	1.45	1.46^{acd}	42.47	42.33	42.40^{a}	29.21 (32.67)	30.81 (33.70)	30.01 (33.19)^{d}
200	5.58	5.31	5.45^{bcde}	1.87	1.84	1.86^{bcd}	1.32	1.39	1.36^{bcde}	42.27	36.75	39.51^{a}	38.10 (38.10)	49.86 (44.90)	43.98 (41.50)^{c}
250	5.44	5.19	5.31^{cdef}	1.87	1.73	1.80^{bcdf}	1.32	1.36	1.34^{cdef}	40.33	36.17	38.25^{a}	47.98 (43.82)	50.60 (45.32)	49.29 (44.57)^{b}
300	5.03	4.67	4.85^{fg}	1.64	1.68	1.66^{fg}	1.25	1.26	1.26^{efg}	40.00	33.17	36.58^{a}	63.13 (52.60)	56.44 (48.69)	59.78 (50.64)^{a}
Mean	5.67^{a}	5.49^{a}		1.89^{a}	1.84^{a}		1.39^{a}	1.45^{a}		44.75^{a}	39.85^{b}		28.93 (29.55)^{b}	31.62 (31.55)^{a}	
CD (5%)	Mutation generations (A) = NS; Doses of gamma rays (B) = 0.49 ; Interaction (A×B) = NS			Mutation generations (A) = NS; Doses of gamma rays (B) = 0.14; Interaction (A×B) = NS			Mutation generations (A) = NS; Doses of gamma rays (B) = 0.14 ; Interaction (A×B) = NS			Mutation generations (A) = 4.83; Doses of gamma rays (B) = NS ; Interaction (A×B) = NS			Mutation generations (A) = 0.89 ; Doses of gamma rays (B) = 1.67 ; Interaction (A×B) = 2.36		

* Figures in parentheses are arcsine values* Different letters in each column are significantly different at P≤0.05 by Duncan's Multiple Range Test (DMRT)

with gamma rays exposure in gladiolus. Sheeshan (1959) concluded that gamma rays result in many leaf and flower abnormalities in gladiolus. Shrivastava *et al.* (2007) in gladiolus and Shukla and Datta (1993) have also reported similar results in chrysanthemum. Moes (1969) treated the corms of gladiolus with gamma rays and observed morphological abnormalities in foliage like narrow leaf, assymetrical development of leaf, leathery texture, notching, sickle shaped leaf etc. Patil (2014) studied in gladiolus and observed that leaf size was reduced significantly and leaves become narrow and leathery.

Mostly variations are non-desirable and we got floral abnormalities such as change in flower shape, flower size, flower form from normal ruffled florets to distorted flower head with different sizes of florets length thus caused floral abnormalities, asymmetrical development of florets and fasciation of floret head. The mean floral abnormalities percentage was maximum (50.64%) in 300 Gy significantly more than all other treatments and minimum (0.00%) in control, irrespective of mutation generations. Among mutation generations, results were significant and maximum floral abnormalities percentage was observed in M_2 generation (31.55%) followed by M_1 generation (29.55%), irrespective of different doses of gamma rays. In the data of interaction between different doses of gamma rays and mutation generations, results were significant. In the present study, floral abnormalities increased with increase in gamma rays doses. At high doses, gamma rays affect genetic material and DNA replication and repair mechanism thus creates variations. These variations may be beneficial or harmful. These results are in conformity with the results of Singh *et al.* (2009) in marigold, where they observed that flower abnormalities percentage of flower increased with increasing radiation dose. Banerji and Datta (1992, 2002, 2003) also reported similar types of floral abnormalities with gamma irradiation in chrysanthemum cultivars.

A significant effect on days to bud appearance, days to full bloom and flowering duration by different doses of gamma rays to marigold was observed (Table 3). The mean days to bud appearance (49.50 days) and days to full bloom (89.83 days) was minimum in control significantly less than all other treatments and maximum mean days to bud appearance (62.50 days) and days to full bloom (106.67 days) was in 300 Gy, irrespective of mutation generations. Among mutation generations for days to bud appearance, results were significant with minimum days taken to bud appearance (53.38 days) in M_1 generation followed by M_2 generation (57.71 days), irrespective of different doses of gamma rays. Among mutation generations for days to full bloom, results were significant and maximum days to full bloom was in M_2 generation (102.10 days) followed by M_1 generations (94.91 days), irrespective of different doses of gamma rays. In the data of interaction, results were non-significant between different doses of gamma rays and mutation generations for days to bud appearance but were significant for days to full bloom. In the present study, the days to bud appearance and full bloom were recorded and data showed increased in days with increase in doses of gamma ray and thus cause delay in flowering. This delay in flowering with higher doses of gamma rays may be attributed to the facts that high ionizing radiations might have led to disruption of biochemical pathways by acting on genes which might have been responsible for triggering the synthesis of some flowering factors. The higher doses of gamma rays inactivate auxins and gibberlins which

Table 3. Effect of gamma rays on days to bud appearance, days to full bloom and flower duration of marigold in two different mutation generations

Treatments (Gy)	Days to bud appearance			Days to full bloom			Flowering duration		
	M_1	M_2	Mean	M_1	M_2	Mean	M_1	M_2	Mean
0 (Control)	48.33	50.67	49.50^{g}	60.33	66.67	63.50^{g}	50.67	60.33	55.50^{fg}
50	49.33	54.00	51.67^{f}	64.33	69.00	66.67^{ef}	51.33	61.00	56.17^{ef}
100	52.67	55.67	54.17^{de}	65.00	71.67	68.33^{de}	52.33	62.67	57.50^{e}
150	51.67	57.00	54.33^{d}	65.67	74.00	69.83^{cd}	54.67	64.33	59.50^{cd}
200	55.00	59.00	57.00^{c}	68.00	74.67	71.33^{bc}	55.33	66.33	60.83^{c}
250	56.67	62.67	59.67^{b}	69.67	75.33	72.50^{b}	59.67	68.66	64.17^{b}
300	60.00	65.00	62.50^{a}	72.00	80.00	76.00^{a}	62.67	71.33	67.00^{a}
Mean	53.38^{b}	57.71^{a}		66.43^{b}	73.05^{a}		55.24^{b}	64.95^{a}	
CD (5%)	Mutation generations (A) = 1.02 ; Doses of gamma rays (B) = 1.90 ; Interaction (A×B) = NS			Mutation generations (A) = 0.91 ; Doses of gamma rays (B) = 1.70 ; Interaction (A×B) = NS			Mutation generations (A) = 0.98 ; Doses of gamma rays (B)=1.83; Interaction (A×B) = NS		

* Different letters in each column are significantly different at P≤0.05 by Duncan's Multiple Range Test (DMRT)

control flowering and it may also activate inhibitors that delay flowering in marigold. The present finding are in conformity with Misra and Mahesh (1993) who reported delay in flowering with increasing doses of gamma radiations in gladiolus. Kumari *et al* (2013) concluded that treatment with higher doses of gamma rays results in delay in emergence of flower bud in chrysanthemum. Dilta *et al.* (2006) in *Dendranthema grandiflora* cv. 'Gulmohar' exposed to gamma rays (Co-60) and bud formation and flowering were delayed by exposure. Singh *et al.* (2009) also observed that day to bloom increased with increase in doses of gamma rays in marigold. Dilta *et al.* (2003) in chrysanthemum, and Srivastava and Singh (2002) in gladiolus observed same results.

The results indicate that with increase in doses of gamma rays, the flowering duration increased cause delay in flowering. The mean flower duration was maximum (67.00 days) in 300 Gy significantly more than all other treatments and minimum (55.50 days) in control and was at par with 50 Gy (56.17 days), irrespective of mutation generation. Among mutation generation, results were significant and maximum flower duration (64.95 days) was observed in M_2 generation followed (55.24 days) by M_1 generation, irrespective of different doses of gamma days. In the data of interaction, results were non-significant between different doses of gamma rays and mutation generations. The delay may be due to decrease in vegetative growth due to higher doses which also effect flowering duration. Kumari *et al.* (2013) studied delayed in flowering at higher doses and at highest dose plant remain at vegetative stage. Datta and Banerji (1993) irradiated rooted cuttings of cv. '*Kalyani Mauve'* with 150, 200 and 250 Gy doses of gamma rays observed delayed flowering behavior. Similar results were also observed in zinnia with treatment using gamma rays by Kole and Meher (2005).

Visual observations includes change in many characters like height of plant, flower shape, flower color, flower size, leaf variegations and development of chimeras were observed keenly and recorded. There was so much variation in plant height, variation from flower size, flower shape (abnormal flower, half flowers, single flowers, flat flowers, male sterile flowers), variations in flower colours, variation in change of leaf colour from light green to darker green colour. The treatment with gamma rays resulted in wide range of colour variations in flower of marigold var. *"Punjab Gainda No.1"* which is originally orange coloured variety with round shape having ruffled florets. The colour changes varies from orange-yellow shade (yellow orange 21 B) to yellow orange (Yellow Orange 15 A) and few yellow shades (Yellow 5 A, Yellow 13 A) flowers were developed by induced mutations created by gamma rays treatment and matched with Royal Horticulture Society Colour Chart (RHS, 1996). In the present study, visual observations observed by gamma rays treatments were similar with previous studies. Mahure *et al.* (2010) irradiated chrysanthemum cv. '*Red Gold'* and found novelties in flower colour induced by the mutagens. Sawangmee *et al.* (2011) observed the effect of gamma rays in the genus *Torenia* to produce a new mutant having yellow coloured flower petals due to disruption of anthocyanins synthesis. Kumari *et al.* (2013) investigated chrysanthemum var. 'Otome Pink' treated with gamma rays and observed leaf abnormalities, one at 10 Gy having yellow colour and other at 15 Gy having quilled petals. Bala and Singh (2015) developed flower mutants with altered or novel flower colour in comparison to original flower colour in *Rosa hybrida* L. cv. *Raktima* with gamma rays. Sisodia and Singh (2015) observed similar results in gladiolus and observed one mutant at 2.5 kR treatment with white flowers, whereas original colour of 'Punjab Dawn' is pink, which is white during VM_1 and it was found stable in VM_2. Patil (2014) resulted in colour variations in florets, with mutants with light colour by 5 KR, bifuricated spike by 6 kR dose in gladiolus. Patil *et al.* (2017) in chrysanthemum cv. "*Local Golden*" irradiated with gamma radiation and floret of original flowers were flat with a small tube at the base where as in case of tubular mutant shape at tip spoon type but the basal portion gave pipe or tube like appearance to the ray florets. Latha and Dharmatti (2018) induced mutation with maximum frequency of chlorophyll mutants (xantha, chlorina, viridis and albina) and morphological mutants were observed in higher doses (500 and 600 Gray) of gamma rays in marigold cv. *Double Orange*.

This shows that gamma radiations caused damaging effect at higher doses and results in change in genetic material to such an extent that so many abnormalities were developed. The mutational abnormalities percentage was more in M_2 generation as compared to M_1 generations. This showed that mutations developed in M_1 generations were stable and it passed down to next generations in M_2 generations and also environment had affected the genetic material and thus results in more abnormalities in M_2 generations. This study showed that gamma radiations are good mutagen to develop variations and thus induced mutations in ornamental crops.

Authors' contribution

Conceptualization of research work and designing of experiments(PS); Execution of field/lab experiments(AC); Analysis of data and interpretation (AC,PS); Preparation of manuscript(AC,PS)

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