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Gamma irradiation effects on photosynthetic pigments and plant morphology of gladiolus (*Gladiolus grandiflorus* L.)

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ABSTRACT
To determine the effect of gamma irradiation on photosynthetic pigments and morphological changes, uniform and healthy corms of eight gladiolus varieties were exposed to different doses of gamma rays viz. 0 (untreated), 25, 40, 55 and 70 Gy from ^{60}Co source. Effect was studied for two generations i.e. M_1 and M_2. Irrespective of varieties, an inversely proportional relationship was recorded between gamma rays dose and photosynthetic pigments in both the generations. Maximum chlorophyll a, chlorophyll b and total chlorophyll content were recorded in untreated plants followed by 25 Gy gamma irradiated plants in both the generations. Carotenoid content was also inversely related to gamma rays dose as maximum carotenoid content (1.36 and 1.50 µg /g in M_1 and M_2, respectively) was recorded in untreated plants and minimum (1.02 and 1.06µg /g in M_1 and M_2, respectively) at 70 Gy. Abnormalities and morphological changes like abnormal leaves (%), blind plants (%) and abnormal spikes (%) were directly related to gamma rays dose and were highest at 70 Gy, irrespective of varieties. Interaction of variety "Purple Flora" with 70 Gy gamma irradiation resulted in maximum percentage of abnormal leaves, abnormal spikes and number of blind plants which shows that amongst all the varieties "Purple Flora" was most responsive to gamma irradiation. Morphological changes were on initial growth as primary effect of gamma rays and the leaves formed later were less abnormal. In general, morphological changes and abnormalities were reduced in M_2 generation as compared to M_1 generation, which shows diminishing effect of gamma rays in successive generations.
Key words: *Gamma irradiation, morphological changes, photosynthetic pigments, mutation, leaf abnormalities*

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INTRODUCTION
Gladiolus is a bulbous ornamental plant which belongs to family *Iridaceae* and well known as commercial cut flower. It is heterozygous and propagated vegetatively by corms which makes it suitable for mutation studies. Gamma rays are ionizing radiations which interact with atoms and molecules to produce free radicals in cell. These radicals can damage or modify important components of plant cells and have reported to affect differently the morphology, anatomy, biochemistry and physiology of the plants depending upon the irradiation levels. The variations in the form of physiological damages, gene mutations and chromosome mutations induced by mutagens in any mutation breeding programme have been used as criteria in determining the mechanism of mutagen action and also the

sensitivity of the biological material towards the mutagenic treatments. It is possible to identify plants, which undergo maximum damage due to irradiation using different types of parameters in different experimental layout. Considering the effects of radiation on plants, the present study was conducted to determine the effects of gamma irradiation on content of photosynthetic pigments and gladiolus morphology.

MATERIAL AND METHODS

Gamma irradiation: Uniform and healthy corms of gladiolus varieties *viz.* Yellow Golden (V_1), Nathan Red(V_2), White Friendship(V_3), Jester Gold(V_4), American Beauty(V_5), Red Majesty(V_6), Purple Flora (V_7)and Algarve(V_8) were irradiated with different doses (0 (untreated), 25, 40, 55 and 70 Gy) of gamma rays at Department of Horticulture, Punjab Agricultural University, Ludhiana. Source of gamma rays was (^{60}Co) Low Dose Irradiator 2000 ANSI- N 433.1. These treated corms along with untreated corms (0Gy) were planted on raised beds under open field condition at Model Floriculture Centre, Pantnagar in randomized block design with factorial concept and each treatment was replicated thrice. The corms harvested from each treatment of M_1 generation were stored in cold store from June-September and in the month of October, planted in the field in the same manner for raising M_2 generation.

Pigment extraction: Chlorophyll content in the leaves was determined by the method of chlorophyll estimation using Dimethyl Sulphoxide (DMSO), devised by Hiscox and Israelstam (4). Freshly removed leaves were finely chopped and 50 mg portion was dipped in a test tube containing 10 ml of DMSO (Dimethyl Sulphoxide). The test tubes were then placed in an oven at 60°C for about two hours to make easy extraction of pigments. The absorbance of the extract was read at 663, 645 and 480 nm. A solution of pure DMSO was used as blank in spectrophotometer (Spectronic 20 D). The pigment content was quantified by using the following equation:

$$\boldsymbol{Chlorophyll\ a} = (12.7\ X\ A663 - 2.63\ X\ A645)X\ (\frac{V}{1000XW})$$

$$\boldsymbol{Chlorophyll\ b} = (22.9\ X\ A645 - 4.48\ X\ A663)X\ (\frac{V}{1000XW})$$

$$\boldsymbol{Total\ chlorophyll} = (20.2\ X\ A645 + 8.02\ X\ A663)X\ (\frac{V}{1000XW})$$

For calculating carotenoid content (C_{X+C}) (µg g^{-1} FW) equation given by Wellburn was applied (19).

$$[\mathbf{CX + C}] = \frac{[1000\ x\ A\ 480 - 2.14\ (Chl\ a) - 70.16\ (Chl\ b)]}{220}$$

Where,
A_{645} = Absorbance at 645 nm wave length
A_{663} = Absorbance at 663 nm wave length
A_{480} = Absorbance at 480 nm wave length
V = Volume of the DMSO (10 ml)
W = Fresh weight of the sample (50 mg)

Morphological characters: Data on different abnormalities and morphological changes were recorded at full bloom stage in M_1 and M_2 and presented in percentage. The data represent the average of the three replicated treatments.

Statistical analysis: Experimental data were subjected to two-way analysis of variance (two-way ANOVA) in Randomized Block Design (RBD) in accordance with the procedure outlined by Gomez and Gomez (1). The transformed values were calculated with the help of square root transformation method.

RESULT AND DISCUSSION

Gamma irradiation effect on photosynthetic pigments:

It is evident from the data (Table 1 and 2) that gamma irradiation had significant effect on photosynthetic pigments. There was gradual decrease in the chlorophyll contents (Chl *a*, Chl *b* and total Chl) with the increase in gamma irradiation dose. Maximum content of Chl *a* (2.38 mg/g) , Chl *b* (0.68 mg/g) and total chlorophyll (3.02 mg/g) was recorded in untreated plants in M_1 whereas minimum content was recorded in 70 Gy gamma irradiated plants (1.65, 0.50, 2.11mg/g Chl *a*, Chl *b* and total chlorophyll, respectively). Similar trend was observed in M_2 generation. In addition, it was observed that the concentration of

chlorophyll *a* was relatively higher than chlorophyll *b* in irradiated and non-irradiated plants. The reduction in chlorophyll *b* may be due to a more selective destruction of chlorophyll *b* biosynthesis or degradation of chlorophyll *b* precursors (7). These results are in conformity with the earlier findings where irradiation reduced chlorophyll content in plants (6). No visually distinct chlorophyll mutant was observed in any variety at any doses of gamma irradiation. These results are also in agreement with earlier findings where chlorophyll was visually insensitive to low doses gamma irradiation (3). Gamma rays belong to ionizing radiation and interact with atoms or molecules to produce free radicals in cells which may leads to reduction in photosynthetic pigments. Photosynthetic pigments can be destroyed by high doses of gamma irradiation, with concomitant loss of photosynthetic capacity (17).

Gamma irradiation effect on carotenoid content:

There was a gradual decrease in the carotenoid content as the dose of gamma rays increased (Table 3). In M_1 generation the difference between carotenoid content of 25 Gy (1.21 µg/g) and 40 Gy (1.16 µg/g) treated plants was not significant and same was the case with 55 Gy (1.07 µg/g) and 70 Gy (1.02 µg/g) gamma rays dose. Although, carotenoid content at all the gamma rays doses was significantly reduced as compared to untreated plants. Also in M_2 generation, maximum carotenoid content was recorded in untreated plants (1.50µg/g) and at highest dose of 70 Gy, the carotenoid content (1.06 µg/g) was significantly reduced as compare to untreated plants. Among varieties, Red Majesty recorded maximum carotenoid content in M_1 and M_2 generation (1.86 and 1.83 µg/g respectively), whereas minimum carotenoid content was recorded in variety Nathan Red in both the generations (0.76 and 0.82 µg/g in M_1 and M_2, respectively). Varieties also differed significantly for carotenoid content. In both the generations lowest level of total carotenoids was obtained in plants irradiated with 70 Gy. These results corroborate the earlier findings where carotenoid content was significantly affected by gamma irradiation and was negatively related with the radiation doses (5,11). Carotenoids are integral constituents of the thylakoid membrane and are usually associated intimately with many of the protein that makes up the photosynthetic apparatus. The light absorbed by the carotenoids is transferred to chlorophyll for photosynthesis and because of this they are called accessory pigments.

Gamma irradiation effect on plant morphology:

Data presented in Table 4 reveal that the leaf aberrations were increased after gamma irradiation as compared to control and were highest at high dose (70 Gy) in both the generations. Among the treatments, maximum number of abnormal leaves (4.25 % in M_1 and 3.04 % in M_2) were recorded in plants treated with 70 Gy gamma rays, which were found significantly higher than the rest of the treatment doses in both the generations. Varietal differences for per cent abnormal leaves were also significant. Variety Jester Gold exhibited significantly higher number of abnormal leaves (3.10 %) than the other varieties but at par with percent abnormal leaves in Purple Flora (2.98%) in M_1 generation, whereas in M_2 generation variety Purple Flora (V_7) exhibited maximum number of abnormal leaves (2.20 %) and was statistically at par with Red Majesty (2.13 %) and Algarve (2.05 %). The interaction of variety Purple Flora with 70 Gy (V_7T_5) resulted in maximum percent abnormal leaves in both the generations (5.26% and 3.95% in M_1 and M_2, respectively). These results are in line with earlier research findings where increased leaf abnormalities were recorded at higher doses of gamma irradiations in chrysanthemum cultivar "Thai Chen Queen" (8). Different types of morphological abnormalities like changes in shape, size, margin, apex fission, fusion and leaf folding were detected at higher gamma rays doses although no case of leaf variegation was recorded (Plate 1a, b, c, d). It is also evident from the Plate (1 a, b, c, and d) that the abnormalities were mostly observed over initial growth as primary effects of gamma rays and the leaves formed later were less abnormal. Furthermore, in M_2 generation the percentage of abnormal leaves was reduced as compare to M_1 generation (Table 4). The increase in percentage of abnormal leaves at higher doses might be due to the reason that high dose irradiation disturbed the synthesis of protein, hormone balance, leaf gas-exchange, water exchange and enzyme activity (2) that could be the cause of the cell death and abnormal morphology. Other researchers also observed deformed leaves, floral sheath variegation, fasciation (12) and narrow leaves, leathery texture (13) after irradiation of gladiolus corms with high doses of gamma rays.

Maximum percentage of blind plants was recorded at the dose of 70 Gy (5.76 %) in M1 generation (Table 5). Irrespective of gamma rays treatment, varieties also differed for this character. Variety "Purple Flora" exhibited maximum percentage of blind plants (3.89 %), which was at par with results in variety Nathan Red (3.56 %) and Jester Gold (3.80 %). Minimum percentage of blind plants was recorded in variety Red Majesty (3.12 %), followed by Yellow Golden (3.28 %), White Friendship (3.56%), American Beauty (3.30 %) and Algarve (3.39 %). In M_2 generation too, maximum percentage of blind plants was recorded at 70 Gy gamma irradiation (5.00%) which was significantly higher than the rest of the treatments. Among the varieties, Jester Gold exhibited maximum percentage of blind plant (3.37 %) and it was at par with Purple Flora (3.34%), White Friendship (3.18%), Nathan Red (3.04 %) and Algarve (2.95 %) and significantly different from Red Majesty (2.41 %) and Yellow Golden (2.68 %). The interaction effect of gamma rays and varieties was found non-significant in both M_1 as well as M_2 generation for this character.

A perusal of data from Table 5 reveals that the percentage of blind plants increased in all the varieties as the dose of gamma rays increased. Though, in M_1 generation percentage of blind plants was more, and reduced in M_2 generation, which might be due to more pronounced effect of ionizing radiation in M_1 and diminishing effect in second year. The results are in agreement with the earlier findings of researchers who recorded that spikes have not produced and plant remained blind at 6 Krad and 7 Krad gamma irradiation in gladiolus (14,16). Kumari *et al.* (10) also recorded in chrysanthemum that at 20 Gy dose some plants remained in vegetative stage and did not produce flowers. The increase in blind plants and no production of spike or if spike emerged there was no production of florets might be due to the fact that in gladiolus emergence of inflorescence always follows production of a definite number of leaves and as a result of exposure to higher dose of gamma rays the number of leaves was reduced drastically which caused non-emergence of inflorescence.

Table 1. Effect of gamma irradiation on Chl *a*, Chl *b* and total chlorophyll content in different gladiolus varieties (M_1)

Photosynthetic pigments																		
	Chl *a* (mg/g)						Chl *b* (mg/g)						Total Chl (mg/g)					
	0 Gy	25 Gy	40 Gy	55 Gy	70 Gy	Mean	0 Gy	25 Gy	40 Gy	55 Gy	70 Gy	Mean	0 Gy	25Gy	40 Gy	55Gy	70 Gy	Mean
V_1	3.08	2.73	2.52	2.34	2.16	**2.57**	0.88	0.73	0.75	0.68	0.60	**0.73**	3.90	3.41	3.23	2.97	2.72	**3.25**
V_2	1.76	1.61	1.53	1.42	1.07	**1.48**	0.37	0.39	0.24	0.24	0.25	**0.30**	2.10	1.97	1.74	1.63	1.30	**1.75**
V_3	1.56	1.48	1.34	1.28	1.20	**1.37**	0.43	0.43	0.43	0.44	0.45	**0.44**	1.97	1.88	1.74	1.69	1.62	**1.78**
V_4	2.57	3.24	3.12	2.62	2.15	**2.74**	0.72	0.97	0.91	0.73	0.61	**0.79**	3.25	4.15	3.97	3.30	2.72	**3.48**
V_5	2.36	2.38	2.12	1.77	1.61	**2.05**	0.72	0.65	0.61	0.49	0.45	**0.58**	3.03	2.98	2.68	2.23	2.03	**2.59**
V_6	3.77	3.60	3.13	2.75	2.28	**3.11**	1.13	1.00	0.87	0.78	0.62	**0.88**	4.82	4.53	3.94	3.49	2.87	**3.93**
V_7	1.55	1.48	1.32	1.11	1.10	**1.31**	0.44	0.50	0.36	0.32	0.43	**0.41**	1.96	1.95	1.65	1.41	1.51	**1.70**
V_8	2.43	1.95	1.86	1.79	1.59	**1.93**	0.78	0.55	0.50	0.44	0.56	**0.57**	3.16	2.47	2.33	2.20	2.13	**2.46**
Mean	2.38	2.31	2.12	1.89	1.65		0.68	0.65	0.58	0.52	0.50		3.02	2.92	2.66	2.37	2.11	

	CD at 5%	SEm±	CD at 5%	SEm±	CD at 5%	SEm±
Gamma Radiation(T)	0.08	0.03	0.06	0.02	0.12	0.04
Varieties (V)	0.11	0.04	0.07	0.03	0.15	0.05
Gamma Radiation * Varieties	0.24	0.08	0.16	0.06	0.34	0.12

V_1- Yellow Golden, V_2 -Nathan Red, V_3-White Friendship, V_4-Jester Gold, V_5-American Beauty, V_6-Red Majesty, V_7-Purple Flora, V_8-Algarve

Table 2.Effect of gamma irradiation on Chl *a*, Chl *b* and total chlorophyll content in different gladiolus varieties (M_2)

Photosynthetic pigments																		
	Chl *a* (mg/g)						Chl *b* (mg/g)						Total Chl (mg/g)					
	0 Gy	25 Gy	40 Gy	55 Gy	70 Gy	Mean	0 Gy	25 Gy	40 Gy	55 Gy	70 Gy	Mean	0 Gy	25Gy	40 Gy	55Gy	70 Gy	Mean
V_1	2.87	2.51	2.95	2.25	2.25	**2.57**	0.85	0.63	0.88	0.64	0.64	**0.73**	3.66	3.09	3.78	2.84	2.85	3.25
V_2	1.80	1.77	1.59	1.49	1.20	**1.57**	0.41	0.34	0.37	0.34	0.25	**0.34**	2.17	2.07	1.93	1.79	1.43	1.88
V_3	1.47	1.60	1.46	1.45	1.17	**1.43**	0.44	0.45	0.42	0.39	0.38	**0.42**	1.89	2.01	1.84	1.81	1.52	1.82
V_4	2.67	3.13	3.41	2.31	2.24	**2.75**	0.75	0.85	0.99	0.62	0.63	**0.77**	3.37	3.92	4.34	2.88	2.83	3.47
V_5	3.05	2.71	2.33	2.21	1.77	**2.42**	0.79	0.70	0.67	0.63	0.48	**0.66**	3.78	3.36	2.96	2.80	2.22	3.02
V_6	3.90	3.59	3.28	2.62	2.45	**3.17**	1.09	1.00	0.96	0.74	0.69	**0.90**	4.92	4.53	4.18	3.30	3.10	4.01
V_7	1.53	1.76	1.62	1.38	1.10	**1.48**	0.37	0.47	0.54	0.38	0.34	**0.42**	1.87	2.20	2.13	1.73	1.42	1.87
V_8	3.23	2.83	2.06	1.79	1.64	**2.31**	0.95	0.81	0.74	0.39	0.66	**0.71**	4.12	3.58	2.77	2.16	2.27	2.98
Mean	2.57	2.49	2.34	1.94	1.73		0.71	0.66	0.70	0.52	0.51		3.22	3.10	2.99	2.42	2.21	

	CD at 5%	SEm±	CD at 5%	SEm±	CD at 5%	SEm±
Gamma Radiation(T)	0.14	0.05	0.07	0.03	0.19	0.07
Varieties(V)	0.18	0.06	0.09	0.03	0.24	0.09
Gamma Radiation * Varieties	0.40	0.14	0.20	0.07	0.54	0.19

V_1- Yellow Golden, V_2 -Nathan Red, V_3-White Friendship, V_4-Jester Gold, V_5-American Beauty, V_6- Red Majesty, V_7-Purple Flora, V_8-Algarve

Table 3.Effect of gamma irradiation on leaf carotenoid content in different gladiolus varieties

Carotenoid content (µg/g)												
	M_1 (2012-13)						M_2 (2013-14)					
	0 Gy (T_1)	25 Gy (T_2)	40 Gy (T_3)	55 Gy (T_4)	70 Gy (T_5)	Mean	0 Gy (T_1)	25 Gy (T_2)	40 Gy (T_3)	55 Gy (T_4)	70 Gy (T_5)	Mean
Yellow Golden (V_1)	1.43	1.67	1.53	1.41	1.32	1.47	1.77	1.50	1.35	1.31	1.42	1.47
Nathan Red (V_2)	0.92	0.74	0.75	0.77	0.62	0.76	0.91	0.79	0.81	0.81	0.76	0.82
White Friendship (V_3)	0.90	0.90	0.81	0.87	0.92	0.88	0.87	0.94	0.83	0.90	0.89	0.89
Jester Gold (V_4)	1.80	1.03	1.26	1.39	1.40	1.38	1.48	1.89	1.02	1.65	1.68	1.54
American Beauty (V_5)	1.40	1.39	1.21	0.91	0.92	1.17	1.72	1.47	1.40	1.29	0.97	1.37
Red Majesty (V_6)	2.39	2.10	1.83	1.59	1.37	1.86	2.40	2.10	1.69	1.77	1.19	1.83
Purple Flora (V_7)	0.88	0.88	0.89	0.66	0.65	0.79	0.86	0.93	0.89	0.84	0.66	0.84
Algarve (V_8)	1.20	1.00	0.98	0.98	0.96	1.02	1.95	1.58	1.10	1.01	0.94	1.31
Mean	1.37	1.21	1.16	1.07	1.02		1.50	1.40	1.14	1.20	1.06	
			CD at 5%		SEm±				CD at 5%		SEm±	
Gamma Radiation(T)			0.08		0.03				0.11		0.04	
Varieties(V)			0.10		0.04				0.14		0.05	
Gamma Radiation * Varieties			0.22		0.08				0.30		0.11	

Table 4. Effect of gamma irradiation on per cent abnormal leaves in different gladiolus varieties

Per cent abnormal leaves												
	M_1 (2012-13)						M_2 (2013-14)					
	0 Gy (T_1)	25 Gy (T_2)	40 Gy (T_3)	55 Gy (T_4)	70 Gy (T_5)	Mean	0 Gy (T_1)	25 Gy (T_2)	40 Gy (T_3)	55 Gy (T_4)	70 Gy (T_5)	Mean
Yellow Golden (V_1)	0.00 (1.00)	2.77 (1.93)	6.00 (2.64)	11.18 (3.49)	17.69 (4.31)	7.53 (2.67)	0.00 (1.00)	0.15 (1.07)	1.47 (1.56)	4.19 (2.23)	5.49 (2.55)	2.26 (1.68)
Nathan Red (V_2)	0.00 (1.00)	0.77 (1.33)	1.85 (1.66)	3.29 (2.07)	7.32 (2.88)	2.65 (1.79)	0.00 (1.00)	0.14 (1.07)	0.25 (1.12)	1.49 (1.58)	3.50 (2.11)	1.08 (1.37)
White Friendship (V_3)	0.00 (1.00)	1.69 (1.63	6.01 (2.65)	9.93 (3.31)	13.74 (3.84	6.28 (2.48)	0.00 (1.00)	0.24 (1.10)	1.63 (1.62)	4.09 (2.25)	7.60 (2.93)	2.71 (1.78)
Jester Gold (V_4)	0.00 (1.00)	2.09 (1.75)	10.74 (3.42)	18.62 (4.43)	23.05 (4.90)	10.90 (3.10)	0.00 (1.00)	0.65 (1.28)	2.45 (1.84)	4.01 (2.23)	9.37 (3.21)	3.30 (1.91)
American Beauty (V_5)	0.00 (1.00)	0.57 (1.24)	1.64 (1.62)	5.14 (2.47)	10.67 (3.41)	3.61 (1.95)	0.00 (1.00)	0.41 (1.18)	1.35 (1.52)	3.27 (2.06)	4.28 (2.29)	1.86 (1.61)
Red Majesty (V_6)	0.00 (1.00)	1.42 (1.55)	5.28 (2.50)	10.03 (3.32)	16.92 (4.23)	6.73 (2.52)	0.00 (1.00)	0.25 (1.11)	3.34 (2.08)	7.20 (2.86)	11.79 (3.58)	4.52 (2.13)
Purple Flora (V_7)	0.00 (1.00)	2.21 (1.79)	6.78 (2.79)	15.82 (4.08)	26.73 (5.26)	10.31 (2.98)	0.00 (1.00)	0.10 (1.05)	2.63 (1.90)	8.44 (3.07)	14.62 (3.95)	5.16 (2.20)
Algarve (V_8)	0.00 (1.00)	1.09 (1.44)	2.97 (1.99)	10.79 (3.43)	25.44 (5.14)	8.06 (2.60)	0.00 (1.00)	0.28 (1.13)	1.89 (1.70)	6.50 (2.73)	12.55 (3.68)	4.24 (2.05)
Mean	0.00 (1.00)	1.58 (1.58)	5.16 (2.41)	10.60 (3.32)	17.70 (4.25)		0.00 (1.00)	0.28 (1.12)	1.88 (1.67)	4.90 (2.38)	8.65 (3.04)	
				CD at 5%		**SEm±**				**CD at 5%**		**SEm±**
Gamma Radiation(T)				0.81(0.12)		0.29 (0.04)				0.46(0.10)		0.17(0.04)
Varieties (V)				1.03(0.15)		0.36(0.05)				0.59(0.13)		0.21(0.05)
Gamma Radiation * Varieties				2.30(0.34)		0.81(0.12)				1.31(0.29)		0.47(0.10)

*Values in parentheses ( ) are square root transformed

Table 5. Effect of gamma irradiation on per cent blind plants in different gladiolus varieties

Per cent blind plants												
	M_1 (2012-13)						M_2 (2013-14)					
	0 Gy (T_1)	25 Gy (T_2)	40 Gy (T_3)	55 Gy (T_4)	70 Gy (T_5)	Mean	0 Gy (T_1)	25 Gy (T_2)	40 Gy (T_3)	55 Gy (T_4)	70 Gy (T_5)	Mean
Yellow Golden (V_1)	0.00 (1.00)	0.00 (1.00)	12.42 (3.62)	27.78 (5.34)	28.52 (5.42)	13.74 (3.28)	0.00 (1.00)	0.00 (1.00)	7.32 (2.89)	11.77 (3.52)	23.90 (4.99)	8.60 (2.68)
Nathan Red (V_2)	0.00 (1.00)	0.00 (1.00)	20.71 (4.63)	28.18 (5.40)	32.22 (5.76)	16.22 (3.56)	0.00 (1.00)	0.00 (1.00)	10.86 (3.43)	20.24 (4.60)	26.11 (5.18)	11.44 (3.04)
White Friendship (V_3)	0.00 (1.00)	0.00 (1.00)	13.03 (3.69)	24.81 (5.07)	34.72 (5.92)	14.51 (3.34)	0.00 (1.00)	0.00 (1.00)	11.96 (3.55)	24.81 (5.07)	26.85 (5.26)	12.72 (3.18)
Jester Gold (V_4)	0.00 (1.00)	8.84 (2.81)	16.67 (4.16)	28.89 (5.43)	30.55 (5.61)	16.99 (3.80)	0.00 (1.00)	6.55 (2.51)	11.27 (3.46)	21.48 (4.64)	26.85 (5.26)	13.23 (3.37)
American Beauty (V_5)	0.00 (1.00)	0.00 (1.00)	15.15	26.06 (5.17)	27.78	13.80 (3.30)	0.00 (1.00	0.00 (1.00)	8.16 (2.74)	13.03 (3.69)	20.74 (4.66)	8.39

			(3.98)		(5.34)		)					(2.62)
Red Majesty (V_6)	0.00 (1.00)	0.00 (1.00)	9.39 (2.90)	23.84 (4.94)	32.22 (5.76)	13.09 (3.12)	0.00 (1.00)	0.00 (1.00)	4.60 (2.21)	11.82 (3.54)	17.78 (4.29)	6.84 (2.41)
Purple Flora (V_7)	0.00 (1.00)	3.03 (1.73)	20.37 (4.54)	33.33 (5.81)	39.81 (6.38)	19.31 (3.89)	0.00 (1.00)	1.75 (1.50)	14.82 (3.87)	24.07 (4.99)	27.78 (5.35)	13.68 (3.34)
Algarve (V_8)	0.00 (1.00)	0.00 (1.00)	14.65 (3.92)	25.76 (5.16)	33.33 (5.86)	14.75 (3.39)	0.00 (1.00)	0.00 (1.00)	10.85 (3.41)	17.97 (4.34)	24.14 (5.00)	10.59 (2.95)
Mean	0.00 (1.00)	1.48 (1.32)	15.30 (3.93)	27.33 (5.29)	32.39 (5.76)		0.00 (1.00)	1.04 (1.25)	9.98 (3.20)	18.15 (4.30)	24.27 (5.00)	
				CD at 5%		**SEm±**				**CD at 5%**		**SEm±**
Gamma Radiation(T)				3.04(0.38)		1.08(0.13)				2.48(0.34)		0.88(0.12)
Varieties(V)				3.84(0.48)		1.36(0.17)				3.14(0.44)		1.11(0.15)
Gamma Radiation * Varieties				NS		3.05(0.38)				NS		2.49(0.35)

Values in parentheses ( ) are square root transformed
NS- Non Significant

Table 6.Effect of gamma irradiation on per cent abnormal spikes in different gladiolus varieties

Per cent abnormal spikes												
	M_1 (2012-13)						**M_2 (2013-14)**					
	0 Gy (T_1)	**25 Gy (T_2)**	**40 Gy (T_3)**	**55 Gy (T_4)**	**70 Gy (T_5)**	**Mean**	**0 Gy (T_1)**	**25 Gy (T_2)**	**40 Gy (T_3)**	**55 Gy (T_4)**	**70 Gy (T_5)**	**Mean**
Yellow Golden (V_1)	0.00 (1.00)	2.78 (1.69)	10.37 (3.02)	14.49 (3.93)	34.92 (5.97)	12.51 (3.12)	0.00 (1.00)	0.00 (1.00)	2.56 (1.65)	6.27 (2.48)	13.69 (3.83)	4.51 (1.99)
Nathan Red (V_2)	0.00 (1.00)	2.78 (1.69)	7.50 (2.66)	26.19 (5.21)	36.51 (6.11)	14.60 (3.34)	0.00 (1.00)	0.00 (1.00)	1.52 (1.45)	8.49 (3.08)	15.74 (4.03)	5.15 (2.11)
White Friendship (V_3)	0.00 (1.00)	5.56 (2.07)	15.37 (3.97)	28.57 (5.32)	36.19 (6.08)	17.14 (3.69)	0.00 (1.00)	2.56 (1.65)	6.48 (2.51)	14.29 (3.91)	15.88 (4.11)	7.84 (2.64)
Jester Gold (V_4)	0.00 (1.00)	2.78 (1.69)	24.07 (5.01)	30.55 (5.61)	33.33 (5.86)	18.15 (3.83)	0.00 (1.00)	0.00 (1.00)	7.04 (2.60)	13.89 (3.85)	21.43 (4.66)	8.47 (2.62)
American Beauty (V_5)	0.00 (1.00)	2.78 (1.69)	3.33 (1.77)	13.23 (3.77)	33.73 (5.86)	10.61 (2.82)	0.00 (1.00)	1.85 (1.52)	5.90 (2.42)	11.20 (3.49)	17.86 (4.26)	7.36 (2.54)
Red Majesty (V_6)	0.00 (1.00)	5.56 (2.07)	16.50 (3.71)	23.15 (4.81)	31.74 (5.72)	15.39 (3.46)	0.00 (1.00)	1.85 (1.52)	7.69 (2.67)	12.22 (3.26)	16.67 (3.73)	7.69 (2.44)
Purple Flora (V_7)	0.00 (1.00)	6.36 (2.50)	26.19 (5.21)	33.97 (5.90)	40.00 (6.40)	21.30 (4.20)	0.00 (1.00)	1.85 (1.52)	7.22 (2.61)	13.69 (3.83)	22.22 (4.76)	9.00 (2.74)
Algarve (V_8)	0.00 (1.00)	5.55 (2.37)	20.74 (4.66)	34.52 (5.95)	33.33 (5.86)	18.83 (3.97)	0.00 (1.00)	0.00 (1.00)	7.42 (2.90)	9.51 (3.24)	13.75 (3.84)	6.14 (2.40)
Mean	0.00 (1.00)	4.27 (1.97)	15.51 (3.75)	25.58 (5.06)	34.97 (5.98)		0.00 (1.00)	1.02 (1.28)	5.73 (2.35)	11.20 (3.39)	17.15 (4.15)	
				CD at 5%		**SEm±**				**CD at 5%**		**SEm±**
Gamma Radiation(T)				3.43(0.54)		1.22(0.19)				2.88(0.51)		1.02(0.18)
Varieties(V)				4.34(0.68)		1.54(0.34)				NS		1.29(0.23)
Gamma Radiation * Varieties				NS		3.44				NS		2.89(0.51)

*Values in parentheses ( ) are square root transformed
NS- Non Significant

Plate 1.Changes in plant morphology as abnormal leaves and spikes, developed after gamma irradiation in different gladiolus varieties

It is evident from the data presented in Table 6 that percentage of abnormal spikes was less at lower doses and increased as the dose increased. Maximum per cent abnormal spikes was recorded at 70 Gy gamma irradiation in M_1 (5.98 %) as well as M_2 generation (4.15 %) which was significantly higher than the untreated plant. Varieties also varied significantly for this character. In M_1 generation, irrespective of gamma irradiation, variety Purple Flora had maximum abnormal spikes (4.20 %) which was at par with Algarve (3.97 %), whereas minimum abnormal spikes were recorded in variety American Beauty (2.82 %), which was at par with White Friendship (3.69%), Nathan Red (3.34 %), Yellow Golden (3.12%) The different shapes (Plate1 e, i and l), bifurcation of spikes (Plate 1g, h, j) and disharmony in floret arrangement (Plate 16 e, f, l, n) was noticed as spike abnormalities and which were

increased after gamma irradiation in all the gladiolus varieties. Similar increase in abnormal flowers was earlier recorded by Kumari *et al* (9).These results are also in close conformity with the findings of Tiwari *et al. (*18), who recorded more number of abnormal spikes, asymmetrical development of florets and fasciation of buds in three gladiolus varieties after gamma irradiation in vM_1 and drastic reduction abnormalities in vM_2 generation and no abnormalities in vM_3 generation. These abnormalities may be due to the fact that at higher doses the growth inhibition induced by the high dose irradiation has been attributed to the cell cycle arrest at G2/M phase during somatic cell division or varied damage in entire genome (15). In general, the percentage of abnormal spikes decreased in M_2 generation as compare to M_1 generation.

CONCLUSION

Exposure of gladiolus corms to different doses of gamma irradiation showed trend of decrease in content of photosynthetic pigments and increase in plant abnormalities with increase in doses. The abnormalities in spikes reverted back to normal growth during a recovery period in M_2 generation in some plants, suggesting the basic cause of abnormalities to be non genetic physiological disturbances in those plants. "Purple Flora" variety was most responsive to gamma irradiation and can be utilized further in mutation breeding programmes.

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