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Morphological variability in *Gladiolus hybridus* L. variety 'Punjab glance' through gamma irradiation

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Abstract
The objective of this study, using gamma rays on gladiolus was to create genetic variability to improve the quantitative traits and to evaluate the genetic variation. The corms of gladiolus variety 'Punjab Glance' were treated with Co^{60} gamma rays at 0, 50, 100, 125 and 150 Gy, and were planted in the field. Data were recorded for various floral parameters. As the dose was increased from 50 to 150 Gy, reduction in spike length, number of florets per spike, floret size, and vase life. Maximum spike length (80.60 cm), number of florets (14.38), floret size (12.34 cm), vase life (15.04cm) were recorded at 50 Gy while the maximum time was taken to initiation of buds (119.30 days) and total days to flowering (120.04 days) was observed with 150 Gy. Flower head fasciation and asymmetrical development of flower heads increased with increased dose of gamma irradiation. Various changes in flower colour and shape were recorded after treatment in the form of chimeras. One mutant having light yellow colour (Yellow Orange 4C) showing slight fading from the original colour was isolated from corm treated with 100Gy were obtained and further multiplied vegetatively.

Keywords: gladiolus, gamma rays, spike length, floret size, floral abnormalities, chimera and mutant.

Introduction
Flowers are the beautiful and greatest gift of God. The very word 'flower' brings in a feeling of a fresh breath. Flower stands as an epitome of all the earthly feelings like love, sacrifice and everything nice. Especially India, the place that is greatly blessed with hundreds of variety flowers of different colours; this makes the country more beautiful and pleasing. Flowers are believed to bring in positive energy. In present scenario floral industry is a dynamic, global fast growing industry which has achieved significant growth rates during the past few decades. India has a blooming future as far as floriculture is concerned. Presently ornamental particularly cut flowers plant seeds and bulbous have a great export potential as well. In recent times floriculture has attained great economic significance and providing a cushion for important avocation and foreign exchange earnings for the country.
Gladiolus (*Gladiolus hybridus* L.) is one of the important bulbous crop, cultivated in various parts of the country. It is an important bulbous crop which occupies important position among cut flowers in domestic as well as international market. It is mainly grown for cut spikes due to wide range of flower colours, longer spikes with good vase life in flower arrangement. The name gladiolus was coined by Pliny the Elder (A.D. 23-79) to describe the shape of the leaf which resembles that of sword (Latin word gladius meaning (sword). It is native to South Africa. The major gladiolus growing countries are Holland, Germany, and the United State of America. In India, the total area under gladiolus cultivation is around 1,270 hectares covering West Bengal, Sikkim, Himachal Pradesh, Jammu and Kashmir, Punjab, Karnataka, Tamil Nadu and Maharashtra. In Punjab, gladiolus is grown in an area of about 150 ha during September to April (NHB, 2014) [21].
Commercial success of any crop depends upon the availability of suitable cultivars to suit the need of the consumers. Physical and chemical mutagens have been observed to be effective tools in creating the variations (Murin *et al.*, 1988) [20]. Mutations are induced in different crops to create variability for further improvement. Mutation is induced by using radiations and other mutagens like EMS, acridine dyes has successfully commercialized a large number of new varieties in different crops including ornamentals plants (Datta, 2009) [6]. Mutagens play an important role in creating new variety in clonal propagated plants because mutations in such plants may easily get stabilized and can be manipulated (Broertjis and Harten, 1978) [5].
Gamma rays are known to influence plant growth and development by inducing cytological, genetical, biochemical, physiological and morphogenetic changes in cells and tissues. Reduction in survival rate, root length, number of spike per plant, floret per spike, days to

flower, shelf life, vase life, floret size, number of corm per plant were observed after exposure to gamma rays at higher doses (Abraham and Desai, 1976, Tiwari *et al.*, 2010) [1, 26]. Induced mutations are highly effective to enhance natural genetic resources for vegetatively propagated crops (Jain, 2006) [14]. Three desirable mutants were obtained in gladiolus exposed to gamma rays (Patil and Dhaduk, 2009) [22]. The main advantage of mutation induction is that the changes can be retained through vegetative propagation. In induced mutation gamma rays have been most successfully used and many new varieties have been developed and released in different ornamentals. In general higher doses were found to be lethal and doses from 0.5 to 5.0 krad are advisable for the gamma irradiation in gladiolus. Thus the present experiment was conducted to study the effect of gamma rays on morphological characters and to evolve mutants for floral and ornamental traits.

Materials and Methods

The present investigation was carried out at the experimental farm of department of Floriculture and landscaping, Punjab Agricultural University from 2014-15 and 2015-2016.The geographical location of PAU is at 30º 54' North (latitude) and 75 º 48 East (longitude) at the height of 247 m above the sea level. Uniform size (4cm) and healthy corms of gladiolus variety 'Punjab Glance' were irradiated with different doses (0,50,100,125, 150 Gy) of gamma rays at College Orchard, Department of Fruit Science, Punjab Agricultural University, Ludhiana using gamma rays Low Dose Irradiator 2000 ANSI-N 433.1. These corms were planted in the field with in 24 hrs of treatment with spacing 30 x 20 cm. The experiment was laid out in Factorial Randomized Complete Block Design with three replications. Observations on spike length (cm), days taken to bud initiation, total days to flowering, number of floret, floret size, vase life, floral abnormalities. During 1st and 2nd years pooled data for all the parameters were statistical analysis was performed using SAS software and treatment means were compared using Duncan Multiple Range Test, (Duncan, 1995) [9].

Results and Discussion

Changes in flowers are one of the important end products of induced mutation. It is evident from the data presented in Table 1 that gamma irradiation significantly delayed the bud initiation. Bud initiation was earlier in control i.e. 83.24 days, which was at par with 50 Gy (87.68 days) while the corms treated with 150 Gy of gamma rays took maximum days i.e. 119.30 days to bud initiation. Out of two years, earlier bud initiation was noticed in first year (99.00 days). It was 102.00 days in second year. As per the results of interaction between gamma rays treatment and years it has been recorded that earlier bud initiation (82.13 days) was observed in untreated corms which were at par with 84.36 days) in second year. The days for bud initiation were increased as the dose of gamma rays increased to 150 Gy (120.15 days) in second year. Delay in bud initiation indicates that it may be due to reduction in the rate of various physiological processes due to gamma rays treatment. This delay initiation and flowering might be due to disturbances in biochemical pathways. Early bud initiation in three cultivars of gladiolus was also reported by (Patil and Dhaduk, 2009) [22]. These results corroborate with the finding of (Datta and Gupta, 1981) [7] in gladiolus where early flowering and bud initiation was reported at lower dose and under control.

Table 1: Effect of gamma irradiation on different floral characters of gladiolus variety 'Punjab Glance'

Doses of gamma rays (Gy)	Days taken to bud initiation (days)			Days to flowering (days)			Spike Length (cm)			Number of florets per spike			Floret Size (cm)		
	vM_1	vM_2	Mean	vM_1	vM_2	Mean	vM_1	vM_2	Mean	vM_1	vM_2	Mean	vM_1	vM_2	Mean
0 (control)	82.13	84.36	83.24 ^{e}	84.43	86.18	85.30 ^{e}	75.09	77.30	76.19 ^{b}	12.41	13.23	12.82 ^{b}	9.36	9.40	9.38 ^{b}
50	88.53	86.83	87.68 ^{d}	90.33	94.18	92.26 ^{d}	80.13	81.07	80.60 ^{a}	13.76	15.00	14.38 ^{a}	12.24	12.44	12.34 ^{a}
100	95.53	99.66	97.59 ^{c}	97.25	98.91	98.08 ^{c}	68.11	67.48	67.79 ^{c}	10.52	11.00	10.76 ^{c}	8.67	8.79	8.73 ^{c}
125	110.37	118.78	114.57 ^{b}	112.89	118.44	115.67 ^{b}	48.66	50.16	49.41 ^{d}	6.13	6.41	6.27 ^{d}	4.93	4.96	4.94 ^{d}
150	118.45	120.15	119.30^{a}	119.70	120.37	120.04 ^{a}	31.83	33.50	32.66 ^{e}	4.59	4.82	4.70 ^{e}	3.93	3.95	3.94 ^{e}
Mean	99.00 ^{b}	102.00 ^{a}	100.47	100.92 ^{b}	103.61 ^{a}	102.27	60.76 ^{b}	61.90 ^{a}	61.33	9.72 ^{b}	10.02 ^{a}	9.87	7.82 ^{b}	7.90 ^{a}	7.86
LSD $^{(p= 0.05)}$	A = 2.83; B = 1.79; A x B = 2.54			A = 12.73; B = 8.05; A x B = NS			A = 1.95; B = 1.23; A x B = 2.25			A = 0.69; B = 0.48; A x B = 0.77			A = 2.83; B = 1.79; A x B = 4.02		

*A = Treatments; B = Year.

The minimum days to flowering were assumed in corms treated at 0 Gy (85.30 days) which was followed by treated corms (92.26 days) at 50 Gy. The more days to flowering was observed at higher doses of gamma rays i.e. 150 Gy (120.04 days). Out of two years, minimum time to flowering was noticed in first year (100.92 days). It was 103.61 days in second year. The results of interaction between gamma rays treatment and years are indicated that earlier flowering (84.43 days) was observed in untreated corms during first year which were followed by 0 Gy (86.18 days) in second year. The days for flowering were increased as the dose of gamma rays increased to 150 Gy (120.37 days) in second year. In general there was decrease in days to flowering with increased dose of gamma rays. Delay in flowering might be due to disturbances in biochemical pathway, which assists in synthesis of flower significantly at 5 KR treatment in various varieties used in their studies. Similar results were also reported by (Kole and Meher, 2005) [16] on the effect of gamma rays on zinnia. These results are in conformity with the observations made by (Singh and Anuj, 2013) [23] who reported beneficial effect of lower doses of gamma rays on various flowering parameters like spike length, floret size and days to flowering.

The results showed significant effect of gamma rays treatment on spike length. The longest spike 80.60 cm was recorded in corms treated with 50 Gy which was at par with untreated corms (76.19 cm) and 100 Gy (67.79 cm). The shortest spike length was observed (32.66 cm) at 150 Gy. Out of two years longest spike was noticed in second year (61.90 cm). It was 60.76 cm in first year. The results of interaction between gamma rays treatment and years are indicated that longest spike (81.07 cm) was observed in treated corms during second year which were followed by 50 Gy (80.13 cm) in first year. The length of spike was decreased as the dose of gamma rays increased to 150 Gy (31.83 cm) in first year. As per the results higher doses effect of gamma irradiation was more pronounced which resulted in smaller spike length and reduced flower size. This might be due to reduction in plant growth. The stimulatory effects observed may be due to to acceleration in the release of certain enzymes or the biological compounds from its bound form to scavenge or due to enhanced biosynthesis of ascorbic acid and sulfhydryl compounds (Flaig and Schmid, 1966) [12]. These results are in close conformity with the findings of (Patil and Dhaduk, 2009) [22].

The results showed significant effect of gamma rays treatment on floret number. The maximum number of floret (14.38) was assumed in corms treated with 50 Gy which was at par with untreated corms (12.82) and 100 Gy (10.76). The number of florets per spike was decreased (4.70) as the doses of gamma ray increased up to 150 Gy. Out of two years maximum number of florets per spike was observed in second year (10.02). The results of interaction between gamma rays treatment and years are indicated that maximum (15.00) was observed in treated corms during second year which were followed by 50 Gy (13.76) in first year. The number of floret was decreased as the dose of gamma rays increased to 150 Gy (4.59) in first year. In general, there was drastic reduction in floret number at highest dose of gamma rays as compared to control. The radiation reduced floret number and affected adversely may be because of auxin destruction, irregular auxin synthesis, failure of assimilation, mechanisms or inhibition of mitotic and chromosomal changes or damage with association of secondary physiological damage. These results are in conformity with findings of (Sisodia and Singh, 2015) [25] who reported that maximum number of florets in control plants and decrease in florets at higher doses of gamma rays in gladiolus.

The results showed significant effect of gamma rays treatment on size of floret. Maximum (12.34 cm) floret size was assumed at 50 Gy which was at par with untreated corms (9.38 cm) and 100 Gy (8.73 cm). Minimum floret size was observed (3.94 cm) at 150 Gy. Out of twoyears, maximum floret size was noticed in second year (7.90 cm). The results of interaction between gamma rays treatment and years are indicated that maximum size of floret (12.44 cm) was observed in treated corms during second year which were followed by 50 Gy (12.24 cm) in first year. The size of floret was decreased as the dose of gamma rays increased to 150 Gy (3.93 cm) in first year. In general, higher doses of gamma rays decrease floret size drastically. Decrease in flower size at higher doses of gamma rays treated was also reported by (Singh *et al.*, 2010) [24] in African marigold. These results are also in conformity with the findings of (Dwivedi and Banerji, 2008) [10] who reported reduction in flower size of Dahlia cv. ‘Pinki’ after gamma irradiation of rooted cuttings.

The results showed significant effect of gamma rays treatment on vase life. Maximum (15.04 days) vase life was assumed at 50 Gy which was at par with untreated corms (14.79 days) and 100 Gy(10.76 days). Minimum vase life was observed (3.98 days) at 150 Gy. Out of two years maximum vase life was noticed in second year (10.01 days). The results of interaction between gamma rays treatment and years are indicated that maximum vase life (15.26 days) was observed in treated corms during second year which were followed by 50 Gy (14.82 days) in first year. Higher doses of gamma rays affected gladiolus varieties ‘Punjab Glance’ and ‘Sylvia’. These results are in close conformity with findings of Dhaduk (1992) who noted that 5 kR and above treatments caused drastic reduction in vaselife of gladiolus cultivars viz. ‘Melody’, ‘Pusa Suhagin’. The flower spike elongated and differentiation of the individual flower occurred as a result of low doses of gamma irradiation in gladiolus (Awad and Harried, 1985) [2]. Karki, 2008 [15] studied the effect of gamma rays on different varieties of gladiolus and reported that the maximum vase life was observed in 1.5 Kr gamma rays treatment and there was decrease in vase life at higher doses *viz.* 2.5 and 3.5 Kr gamma rays.

Abnormalities

As a result of gamma rays treatment different morphological abnormalities was recorded in gladiolus variety ‘Punjab Glance’. The data on different parameters like asymmetrical development of spike, bud and floret fasciation, abnormal spike (%) and floral abnormalities were recorded to study the abnormalities. The results obtained in respect of these parameters are presented and discussed in Table 2.

Table 2: Effect of gamma irradiation on different floral abnormalities of gladiolus variety ‘Punjab Glance’

Doses of gamma rays (Gy)	Asymmetrical development of spike			Bud and Floret fasciation			Anormal spike (%)			Floral abnormalities (%)			Vase life (days)		
	vM1	vM2	Mean	vM1	vM2	Mean	vM1	vM2	Mean	vM1	vM2	Mean	vM1	vM2	Mean
0 (control)	0.00 (1.00)	0.00 (1.00)	0.00 (1.00) e	0.00 (1.00)	0.00 (1.00)	0.00 (1.00) e	0.00 (1.00)	0.00 (1.00)	0.00 (1.00) e	0.00 (1.00)	0.00 (1.00)	0.00 (1.00) e	14.74	14.85	14.79 b
50	43.33 (6.59)	41.66 (6.43)	42.49 (6.51) d	13.56 (3.81)	14.19 (3.89)	13.87 (3.85) d	39.33 (6.32)	44.33 (6.72)	41.83 (6.52) d	52.96 (7.34)	55.26 (7.50)	54.11 (7.42) d	14.82	15.26	15.04 a
100	72.04 (8.54)	71.33 (8.50)	71.68 (8.52) c	29.04 (5.47)	32.26 (5.76)	30.65 (5.61) c	47.29 (6.94)	47.96 (6.99)	47.62 (6.96) c	62.44 (7.96)	63.78 (8.04)	63.11 (8.00) c	10.49	11.03	10.76 c
125	82.15 (9.11)	81.45 (9.08)	81.80 (9.09) b	35.08 (5.99)	39.41 (6.35)	37.24 (6.17) b	67.41 (8.27)	68.59 (8.34)	68.00 (8.30) b	78.74 (8.92)	78.78 (8.93)	78.76 (8.92) b	4.63	4.95	4.79 d
150	92.04 (9.64)	91.11 (9.59)	91.57 (9.62) a	46.11 (6.86)	47.52 (6.96)	46.81 (6.91) a	80.82 (9.04)	82.37 (9.13)	81.59 (9.08) a	81.70 (9.09)	83.37 (9.18)	82.53 (9.13) a	3.96	4.00	3.98 e
Mean	57.91 (6.97) a	57.11 (6.92) b	57.50 (6.94)	24.75 (4.62) b	26.67 (4.79) a	25.71 (4.70)	46.97 (6.31) b	48.65 (6.43) a	47.80 (6.37)	55.16 (6.86) b	56.23 (6.93) a	55.70 (6.89)	9.72 b	10.01 a	9.87
LSD (p= 0.05)	A = 0.36; B = 0.25; A x B = 0.40			A = 0.12; B = 0.07; A x B = 0.11			A = 0.25; B = 0.16; A x B = 0.22			A = 0.12; B = 0.07; A x B = 0.11			A = 0.48; B = 0.34; A x B = 0.54		

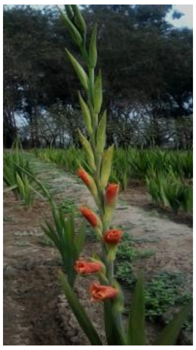
*A = Treatments; B = Year. Figures in parentheses are square root transformed values

Maximum number of plants with asymmetrical spikes (9.62 %) was recorded at 150 Gy which was at par with 125 Gy (9.09 %) and 100 (8.52 %). Spikes were decreased significantly at 0 Gy of gamma rays treatment (1.00%). Out of two years, minimum number asymmetrical spikes were recorded in variety second year (6.92 %). The results of interaction between gamma rays treatment and varieties indicate that the minimum (1.00 %) asymmetrical spikes were recorded at 0 Gy in both years. Asymmetrical spikes increased to (9.64 %) as the doses were increased to 150 Gy in first year. As per the observation the formation of spikes and development of floret was affected due to gamma rays. Asymmetrical development of spike might be also due to chromosomal aberrations, disturbance in the production and or distribution of growth substances caused by gamma rays as reported by (Gunckel, 1957) [13]. Present findings are also corroborated with the experimental studies of (Misra and Bajpai, 1983 and Misra, 1983) [18, 17] treated gladiolus varieties viz., 'Blue Lilac', 'Himprabha', 'Jo-Wagenaar', 'Picardy, 'Ratna's Butterfly', 'Sans Sauci', 'Snow Princess' and 'Sylvia' at 3, 4, 5, 7 and 10 kR doses of gamma rays found asymmetrical spikes.

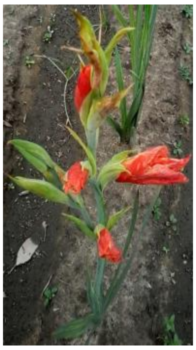
It is evident from the data that significant increase was found in percent plants with fasciation of bud due to gamma ray treatment (Fig 1). Minimum number of plants with bud fasciation (1.00 %) was recorded at 0 Gy which was at par with 50 Gy (3.85 %) and 100 Gy (5.61 %). Bud fasciation was increased significantly at 150 Gy gamma rays treatment (6.91 %). Out of two years, minimum bud fasciation was recorded in first year (4.62 %). The results of interaction between gamma rays treatment and varieties indicate that the minimum (1.00 %) asymmetrical spikes were recorded at 0 Gy in both years. Asymmetrical spikes increased to (6.96 %) as the doses were increased to 150 Gy in second year. As per the observation the bud and floret fasciation was affected due to gamma rays. This abnormality might be also due to chromosomal aberrations, disturbance in the production and or distribution of growth substances caused by gamma rays as reported by (Gunckel, 1957) [13]. The formation of fascinated heads after irradiation are in close conformity with the findings of (Banerji and Datta, 1992) [3] and (Dwivedi *et al.*, 2000) [11]. Maximum abnormal spikes (9.11 %) was recorded at 150 Gy which was at par with 125 Gy (8.34 %) and 100 Gy (6.95 %).

Abnormal spikes were decreased significantly at 0 Gy gamma rays treatment (1.00%).Out of two years minimum number abnormal spikes was recorded in first year (6.31%). The results of interaction between gamma rays treatment and varieties indicate that the minimum (1.00 %) abnormal spikes were recorded at 0 Gy in both years. Abnormal spikes increased to (9.13 %) as the doses were increased to 150 Gy in second year. These abnormalities may be due to the fact that higher doses have many mutational events which may be induced with increase risk of favorable (Donini, 1975) [8]. Spike abnormalities were noticed after gamma irradiation at higher doses. These results are in close conformity with the findings of (Tiwari *et al.*, 2010) [26] who recorded more number of abnormal spikes in three gladiolus varieties after irradiation. Morphological abnormalities in the foliage and florets were also observed in the irradiated material of gladiolus (Banerji and Datta, 1998) [4].

Maximum floral abnormalities (9.13 %) was recorded at 150 Gy which was at par with 125 Gy (8.92 %) and 100 Gy (8.00 %). Floral abnormalities were decreased significantly at 0 Gy gamma rays treatment (1.00%). Out of two years minimum floral abnormalities was recorded in first year (6.86 %). The results of interaction between gamma rays treatment and varieties indicate that the minimum (1.00 %) floral abnormalities were recorded at 0 Gy in both years. floral abnormalities of spikes increased to (9.18 %) as the doses were increased to 150 Gy in second year. This abnormality might be due to chromosomal aberrations, disturbance in the production and/or distribution of growth substances caused by gamma rays. The formation of fascinated bud and floret after irradiation are in close conformity with the findings of (Misra *et al.*, 2009) [19]. As a result of gamma rays treatment chimera was obtained in 'Punjab Glance' (Fig 2). Bicoloured floret was observed at 100 Gy in 'Punjab Glance'. One sectorial chimera was observed on the lower portion of the floret at 100 Gy dose. Most of petals were broken and very light in colour than original variety was noticed at higher dose. Morphological, palynological and anatomical characters of mutant developed after gamma ray treatment in variety 'Punjab Glance'. The observations were recorded on morphological, palynological and anatomical characters of mutant developed after gamma rays treatment in variety 'Punjab Glance' is presented in Table 3. One mutant, M-1 was developed in variety 'Punjab Glance'.



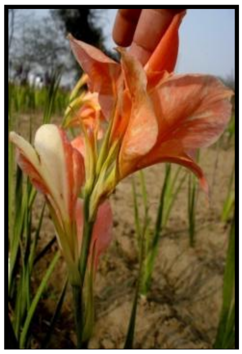
Normal Spike



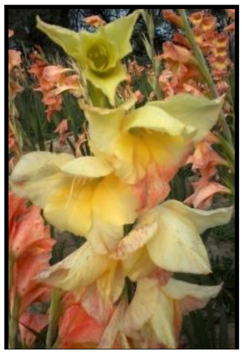
Asymmetrical Floral Spike100Gy

Fasciation of bud at100Gy

Fig 1: Floral abnormalities



Chimera (bicoloured floret) at 100 Gy



M-1 at 100Gy (Yellow Orange 4C)

Fig 2: Flower colour mutations (chimeras) bicolored floret, Mutant screened in M-2

Table 3: Morphological, palynological and anatomical characters of mutant developed after gamma rays treatment in variety 'Punjab Glance'

Characters	Punjab Glance	Mutant (M-1) 100 Gy
Sprouting of corms (%)	99.16	95.00
Days taken to sprouting (days)	16.66	15.00
Plant height (cm)	97.21	99.00
Number of leaves	5.00	7.00
Days to bud initiation (days)	83.24	82.00
Total days to flowering (days)	93.95	95.67
Spike length (cm)	77.24	78.00
Number of florets/ spike	14.33	15.00
Floret size (cm)	9.38	10.50
Length of leaf (cm)	55.00	39.00
Width of leaf (cm)	2.27	1.70
Length of pistil (cm)	7.89	9.50
Weight of spike (g)	25.16	24.00
Vase life (days)	12.00	14.00
Pollen fertility (%)	91.96	93.67
Number of stomata (mm^2)	15.29	17.00
Size of stomata (μm)	25.36	25.50
Corms per plant	2.00	2.00
Cormels per plant	28.00	28.79
Weight of corms per plant (g)	51.30	49.00
Weight of cormels per plant (g)	9.59	9.00
Corm size (cm)	4.94	4.00
Leaf abnormalities (%)	0.00	50.00
Floral abnormalities (%)	0.00	60.00
Flower colour(R.H.S)	Orange group 24 B	Yellow Orange 16 A

Conclusion
From the present study it was concluded that at 100 Gy were found to be optimum for the induction of mutagenesis under *in vivo* in gladiolus variety 'Punjab Glance'. It has shown that using radiations in ornamental crops has come to stay as an efficient plant breeding method and useful tool for developing new varieties.

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