Journal of Pharmacognosy and Phytochemistry
Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
www.phytojournal.com
JPP 2020; Sp 9(4): 227-233
Received: 16-05-2020
Accepted: 18-06-2020

Kiran Kumari
Assistant Professor, Department of Floriculture & Landscape Architecture, College of Horticulture, S.D. Agricultural University, Jagudan, District. Mehsana, Gujarat, India

Santosh Kumar
Professor, Department of Horticulture (Floriculture and Landscaping), G.B. Pant University of Agriculture and Technology, Pantnagar, Uttarakhand, India

Corresponding Author:
Kiran Kumari
Assistant Professor, Department of Floriculture & Landscape Architecture, College of Horticulture, S.D. Agricultural University, Jagudan, District. Mehsana, Gujarat, India

Frequency and spectrum of flower colour mutations in gamma irradiated gladiolus varieties

Kiran Kumari and Santosh Kumar

Abstract
Gladiolus, the queen of bulbous ornamentals is very popular perennial cut flower owing to its marvelous beauty, striking colours, various sizes and shapes of flower, variable spike length and long vase life. In this experiment corms of eight varieties of gladiolus (Yellow Golden, Nathan Red, White Friendship, Jester Gold, American Beauty, Red Majesty, Purple Flora and Algarve) were treated with gamma rays and the frequency and spectrum of morphological mutations observed up to three generations at Model Floriculture Centre, G.B. Pant University of Agriculture and Technology during2012-2015.
Uniform and healthy corms of eight gladiolus varieties were taken and irradiated with different doses (0, 25, 40, 55 and 70 Gy) of gamma rays from ^{60}Co source and planted in open field condition in Randomized Block Design (RBD) with factorial concept. The outcomes indicated that the plant survival decreased after gamma irradiation and minimum survival (77.11%) was recorded at 70 Gydose. Differences in varietal response to different mutagen doses were clearly evident as all the varieties differed in the spectrum and frequency of induced mutations. In vM_1generation maximum flower colour mutation frequency was recorded in variety Purple Flora followed by Algarve and White Friendship. Irrespective of varieties, among different doses maximum mutation frequency was recorded at 55 Gy. In vM_1 generation, maximum mutation frequency (18.52%) was recorded in Purple Flora variety at 55 Gy gamma rays irradiation. Most of the mutants were in the form of chimera in vM_1 and few disappeared in vM_2 whereas most of the mutants isolated in vM_2 generation were solid mutants. A wide spectrum of colour was observed in treated plants and in general variants were lighter in colour than the original variety. Sixteen mutants, isolated from three varieties of gladiolus (White Friendship, Purple Flora and Algarve) were planted to raise vM_3generation and out of these twelve were found stable for their colour.

Keywords: Gladiolus, gamma rays, mutation spectrum and frequency, mutants

Introduction
Gladiolus is a perennial cut flower known for its tall spikes &large, colorful blooms. It is one of the leading geophytes grown worldwide and looks spectacular in bouquets and other flower arrangements. Gladiolus is highly heterozygous in its genetic constitution which makes it promising material for induced mutagenesis. Induced mutagenesis has great potential as a complimentary approach in genetic improvement of cropsbecause it provides a spectrum of variants for effective and better selection (Dhumal and Bolbhat, 2012) [4]. Exposing the genetic materials to mutagenic agents bring changes in nuclear DNA and/or cytoplasmic organelles which results in genomic or chromosomal mutations enabling the plant breeders to select useful mutants. In any mutation breeding plan for the production of high frequency of desirable mutation, selection of an effective and efficient mutagen is indispensable (Roychowdhury and Tah, 2011) [11].
Gamma rays are electromagnetic radiations of short wavelength and extremely high frequency. High energy per photon along with this accurate dosimetry, reasonable reproducibility, and high and uniform penetration in multicellular system make gamma rays a suitable physical mutagen. Keeping these facts in mind this experiment was carried out to find out spectrum and frequency of colour variants in gamma rays treated gladiolus varieties

Material and methods
Experiment was conducted under open field condition at Model Floriculture centre, G.B. Pant University of Agriculture and Technology for three successive generations during the year 2012-2015. Uniform and healthy corms of eight varieties of gladiolus (Yellow Golden, Nathan Red, White Friendship, Jester Gold, American Beauty, Red Majesty, Purple Flora and Algarve) were irradiated with different doses (0, 25, 40, 55 and 70 Gy) of gamma rays from ^{60}Co source. Experiment was laid out in Randomized Block Design. Survival rate was calculated by using below mentioned formulato know the sensitivity of varieties in vM_1generation.

Plant survival (%) = Number of corms / Number of plant survivedX100

The corms harvested from vM_1were planted to raise vM_2, andfrom vM_2 only selected mutants were planted to raise vM_3 generations to evaluate qualitative and quantitative characters. Flower colour mutation frequency and the spectrum of colour were recorded in all the three generations. Colour was recorded by Royal Horticultural Society Colour Chart (R.H.S. Colour Chart) whereas frequency of flower colour mutation was estimated by using following formula:

$$\text{Mutation frequency (\%)} = \frac{\text{Number of flower colour mutants}}{\text{Total number of plants scored}} \times 100$$

Result and discussion

Plant survival (%)

It is evident from the data (Table 1) that plant survival decreased significantly after gamma rays irradiation, irrespective of varieties used. There was 100 per cent survival in untreated plants ofall the varieties. Survival percentage was 98.62 per cent at 25 Gy dose, which was at par with survival percentage in untreated plants, whereas further increase in dose to 40 Gy (89.62%), 55 Gy (83.03%) and 70 Gy (77.11%), significantly reduced plant survival per cent. Varietal differences for radiation sensitivity were also highly significant. Among the varieties, Purple Flora was found to be more sensitive to higher exposure (84.46% survival) followed by Jester Gold (86.13%) and Red Majesty (88.91%), whereas Nathan Red (93.36%), American Beauty (91.69%), Algarve (91.13%) and Yellow Golden (90.58%) varieties were significantly more tolerant to gamma irradiation than the earlier three varieties. Interaction of variety Purple Flora with 70Gy gamma rays resulted in minimum survival (69.47%) which was at par with survival in Jester Gold at 70 Gy (72.23%).Differences for radiation sensitivity among cultivars were also reported by other workers (Broertjes and Van Harten, 1988) [2]. Survival of irradiated material in vM_1 generation is considered as one of the important criteria to estimate the dose levels for particular mutagen. When death rate reaches 50 per cent of total treated material (LD_{50})such dose or concentration is considered as optimum one. In this experiment LD 50 was found beyond 70 Gy. Gamma rays are ionising radiations and ionize water molecules. The exposure of a biological system to ionizing radiations activates a number of physical and chemical steps between the initial absorption of energy and the final biological injury. In biological tissues, these ionizations are induced all along the radiation path, leading to chain reactions, which produce secondary reactive oxygen species (ROS) as a result of $H^+$and e^-_{aq} becoming trapped (Esnault *et al*., 2010) [6]. These radicals can damage or modify important components of plant cells and affect certain physiological and biochemical processes which are vital for survival and that might be the reason for reduction in survival percentage after irradiation.

Table 1: Effect of gamma irradiation on plant survival in different gladiolus varieties

Plant survival (%)						
vM_1						
	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)	100.00	100.00	91.70	80.60	80.60	90.58
Nathan Red (V_2)	100.00	100.00	94.47	88.93	83.40	93.36
White Friendship (V_3)	100.00	100.00	91.70	86.17	77.80	91.13
Jester Gold (V_4)	100.00	97.23	83.40	77.80	72.23	86.13
American Beauty (V_5)	100.00	100.00	91.70	86.17	80.60	91.69
Red Majesty (V_6)	100.00	94.47	88.93	83.37	77.80	88.91
Purple Flora (V_7)	100.00	97.23	80.60	75.00	69.47	84.46
Algarve (V_8)	100.00	100.00	94.47	86.17	75.00	91.13
Mean	100.00	98.62	89.62	83.03	77.11	
	CD at 5%			S.Em±		
Gamma Radiation	2.08			0.74		
Varieties	2.63			0.93		
Gamma Radiation * Varieties	5.88			2.08		

Frequency and spectrum of flower colour mutations in vM_1generation

It is evident from the data (Table 2) that flower colour mutation frequency is genotype dependent as different varieties shows difference in frequency and spectrum under same exposure. Irrespective of varieties, at 25 Gy dose there was no or very less flower colour changes.

At 25 Gy dose, there wereno flower colourvariants recorded in varieties Yellow Golden, White Friendship and American Beauty. Also at 70 Gy dose, there were no flower colourvariants recorded in Yellow Golden, Nathan Red, American Beauty and Red Majesty. Mutation frequency was less in varieties Yellow Golden, Nathan Red, Jester Gold, American Beauty and Red Majesty; whereas it was recorded highest in Purple Flora followed by Algarve and White Friendship. Among all the varieties, Purple Flora had maximum flower colour mutation frequency (18.52%) at 55 Gy gamma rays dose. Further, it is also evident that flower colour mutation frequency increased as the dose of gamma rays increased upto 55Gy but at 70Gy again a reduction in flower colour mutation frequency was recorded. These results are in close conformity with the findings of Rather *et al*. (2002) [10], who reported increase in mutation frequency at higher doses of gamma irradiation in Dutch Irish. At highest dose (70 Gy) lethality as well as the number of blind plants/ unopened florets/ fussed florets was more in vM_1 generation, which might be the reason for reduction in colour variants at this dose as compared to the 55 Gy in different gladiolus varieties.

Similar to mutation frequency, the spectrum of mutations also varied with cultivars. In vM_1 generation, most of the variants were in the form of chimeras and the size of mutated sector varied from variety to variety from narrow streak on petal to entire petal, a single floret on spike to more than one floret to whole spike (Plat 1). Two or more colours were observed on a singlefloret or on single spike.In general variants were lighter in colour than the original variety Flower colour is related with different pigment formation pathways and because every variety had a different colour so their response to colour change was different. These results are in parallel line with the findings of Kumari *et al.* (2013) [7], who also reported such type of sectorial chimeras in vM_1 generation of gamma irradiated chrysanthemum. It is clear that radio sensitivity in different ornamentals is a genotype dependent mechanism. The frequency of mutation varied with the varieties and dose of gamma rays. Some varieties were more sensitive, others responded moderately, and some were very less responded towards mutagen exposure especially for flower colour.

Table 2: Effect of gamma irradiation on flower colour mutation frequency in vM_1 generation of gladiolus

Variety	Gamma rays dose	Plants evaluated	Flower colour mutants	Spectrum	Flower colour mutation frequency (%)
Yellow Golden	25 Gy	36	-	-	0.00
	40Gy	32	1	Green YellowGroup8C	3.12
	55 Gy	29	1	Yellow Group3B+OrangeRed Grooup29A	3.45
	70Gy	28	-	-	0.00
Nathan Red	25 Gy	36	1	Red Purple Group57C+Red Group33D	2.77
	40Gy	34	1	White Group 155C	2.94
	55 Gy	32	-	-	0.00
	70Gy	28	-	-	0.00
White Friendship	25 Gy	35	-	-	0.00
	40Gy	31	2	Red Group39D	6.45
	55 Gy	28	2	Red Group39D	7.14
	70Gy	26	1	Red Group39D	3.85
Jester Gold	25 Gy	35	-	-	0.00
	40Gy	30	-	-	0.00
	55 Gy	28	1	Yellow Group 8C+Red Group 39B	3.57
	70Gy	26	1	Green Yellow Group 1C	3.85
American Beauty	25 Gy	35	-	-	0.00
	40Gy	33	1	Orange Red Group30A	3.03
	55 Gy	31	1	Red Group48C	3.23
	70Gy	29	-	-	0.00
Red Majesty	25 Gy	34	1	White Group 155 D	2.94
	40Gy	32	1	White Group 155 D	3.12
	55 Gy	30	-	-	0.00
	70Gy	28	-	-	0.00
Purple Flora	25 Gy	34	2	Purple Group75D+Red Purple Group57C, Purple Violet Group77C	5.88
	40Gy	29	2	Purple Group82A+ Red PurpleGroup57C, RedPurpleGroup57C+PurpleGroup 70B	6.90
	55 Gy	27	5	Purple Group82A+Red Purple Group57C, Violet Blue Group77D+Purple Group80A, Purple Group82A+red Purple Group57C, Purple Group84A+Red Purple Group57C, Violet Blue Group87D	18.52
	70Gy	25	3	PurpleGroup81A+White Group 155D, PurpleGroup82A+White Group 155C, Purple Violet Group81D	12.00
Algarve	25 Gy	36	1	Red Group36A	2.77
	40Gy	34	2	Red Group 33C+Red Group36A, Orange Red Group30A	5.88
	55 Gy	31	4	Yellow Group 4B +Red Group 37C, Red Group 34A+ Red Group 49C, Red Group34C+Yellow Group 8A	12.90
	70Gy	27	3	Orange Red Group 29C+Green Yellow Group 1C, Red Group 37C, Orange Red Grouop 33D+Green Yellow Group 4C	
Total			37		

Frequency and spectrum of flower colour mutations in vM_2 generation

The data on the mutation frequencies and spectrum in vM_2 generation are summarized in Table 3.It is evident from the data that the mutation frequency was decreased in vM_2 generation as compared to vM_1. Highest mutation frequency was recorded in variety Purple Flora at 55 Gy (10.00%) followed by mutation frequency at 70 Gy in the same variety (7.69%). Flower colour mutation frequency in Nathan Red at 40 Gy was 1.51% and in variety White Friendship 2.94% and 3.57% at 40 and 55 Gy, respectively. In variety Algarve, mutation frequency was 2.13%, 5.00% and 6.90% at 40, 55 and 70 Gy, respectively. No flower colour mutation was recorded in variety Yellow Golden, Jester Gold, American Beauty and Red Majesty at any irradiation dose in vM_2 generation. Most of the mutations noticed in vM_1 generation were not observed again in vM_2 generation. This disappearing of chimeric mutants might be due to the diplontic selection Total sixteen mutants were isolated, two from Nathan Red, three from White Friendship, six from Purple Flora and five from Algarve (Plate 2). All mutants had different floret colour than the original varieties and in most of the cases colour was lighter as compare to original varieties.

In comparison to colour variants recorded in vM_1 generation, variants that were recorded in vM_2 were having larger chimeric sector size and most of the plants had wholly mutated spikes. These results are in conformity with the work of Sood and Pathania (2007) [12], Girija *et al*. (2013) [8] and Bhat *et al.* (2005) [3], who recorded decreased mutation frequency in vM_2 generation. The results are also in agreement with the findings of Dhaduk *et al*. (1992) [5], who isolated five flower colour mutants from gamma rays irradiated corms of four varieties of gladiolus and explained that flower colour was associated with corresponding change in anthocyanin content. Reduction in anthocyanin pigments in the petals was explained due to certain disturbances in the synthesis of these pigments. It is also suggested by some workers that several cycles of propagation are needed to obtain homo-histons or to dissolve chimeras and to obtain solid mutants in vegetatively propagated plants after mutagen treatment (Ahloowalia and Maluszynski, 2001) [1].

Table 3: Effect of gamma irradiation on flower colour mutation frequency in vM_2 generation of gladiolus

Variety	Gamma rays dose	No. of plants evaluated	Flower colour Mutants	Flower colour spectrum	Flower colour Mutation Frequency (%)
Nathan Red	25 Gy	80	1	Red Purple GroupN74	1.25
	40Gy	66	1	Red Purple Group63C	1.51
	55 Gy	45	-	-	0.00
	70Gy	36	-	-	0.00
White Friendship	25 Gy	47	-		0.00
	40Gy	35	1	Green Yellow Group1C	2.86
	55 Gy	28	1	Red Group39D	3.57
	70Gy	26	1	Red Group39D	3.85
Purple Flora	25 Gy	62	1	Purple Violet Group77C	1.61
	40Gy	42	-	-	0.00
	55 Gy	30	3	Violet Blue Group87D, Violet Group86C, Violet Group 86D+VioletGroup86B	10.00
	70Gy	26	2	Violet Group92A	7.69
Algarve	25 Gy	54	-	-	0.00
	40Gy	47	1	Orange Red Group30 A	2.13
	55 Gy	40	2	Red Group47C+RedGroup33C, Orange Red Group27B+Yellow Group3B	5.00
	70Gy	29	2	Orange Red Group29C+Green Yellow Group1C, Orange RedGroup27C	6.90
Total			16		

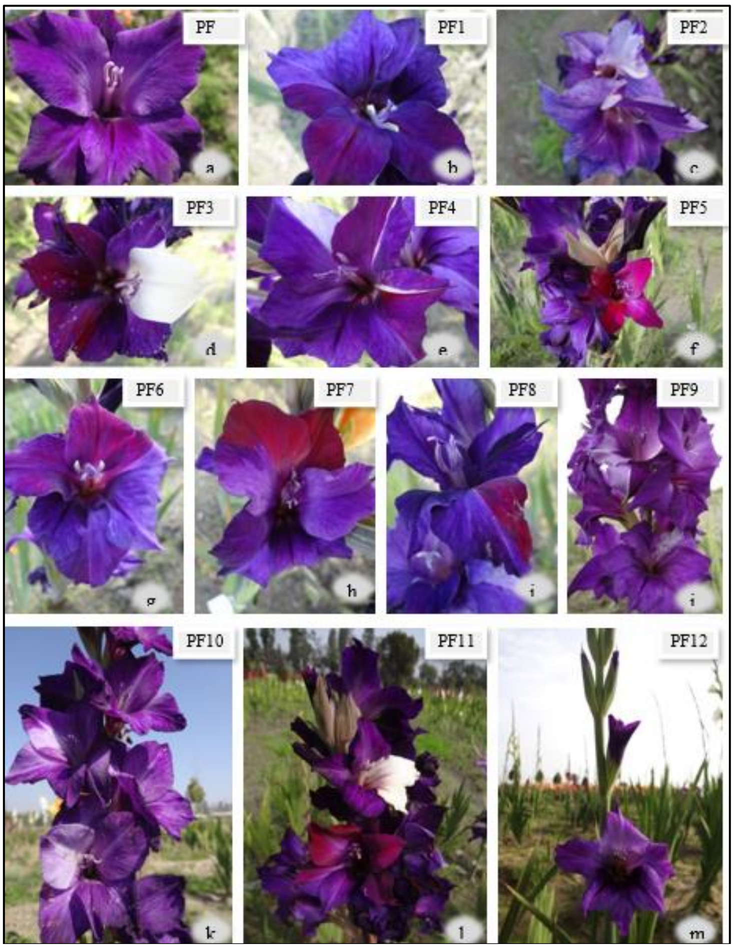

Plate 1: Flower colour mutations (chimeras) in vM_1 generation of variety Purple Flora

Screening of mutants in vM_3 generation

Total sixteen mutants were planted in vM_3 generation to study their stability and most of them were found to be stable, but two mutants of Nathan Red variety were blind and did not produce spike. This might be due to the reason that corm size was reduced or may be some physiological disturbances. Twelve mutants were stable till the completion of vM_3 generation (Plate 3). It is well apparent that flower colour is controlled by many genetic and physiological factors. These factors modify the pigment and produce co-pigment or change the cell condition in such a way so as to produce a wide range of one type of pigment. Exposure to ionizing radiation had caused little disturbance in pigment synthesis might be the reason for different shades of floret colours in gladiolus varieties but stable and solid mutants might be due to changes at DNA level. The reversion of some mutants in the original colour shows that changes were merely due to physiological disturbances caused as a result of irradiation.

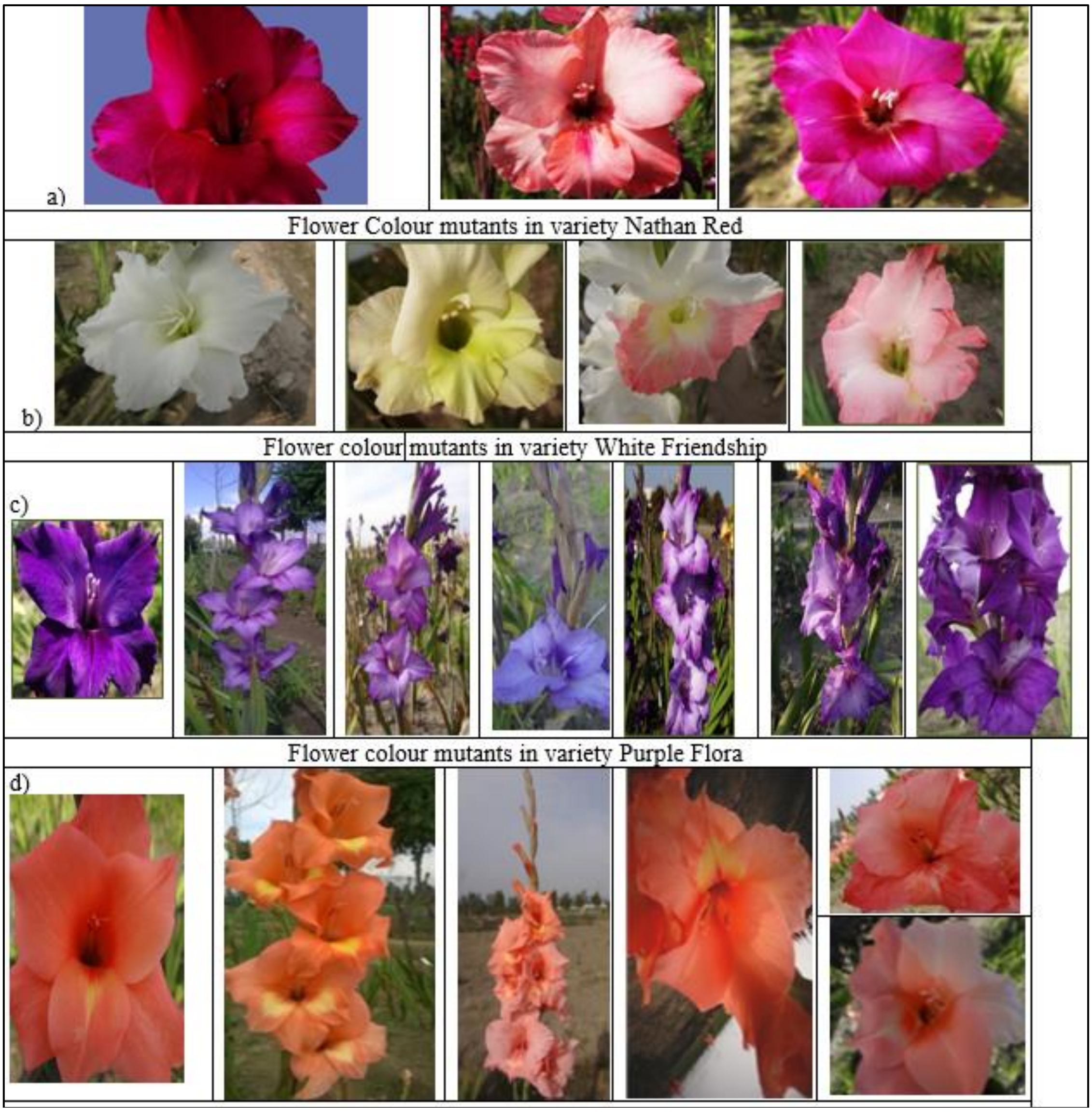

Plate 2: Flower colour mutants in $vM2$ generation of gamma irradiated gladiolus varieties
a) Nathan Red b) White Friendship c)Purple Flora d) Algarvae

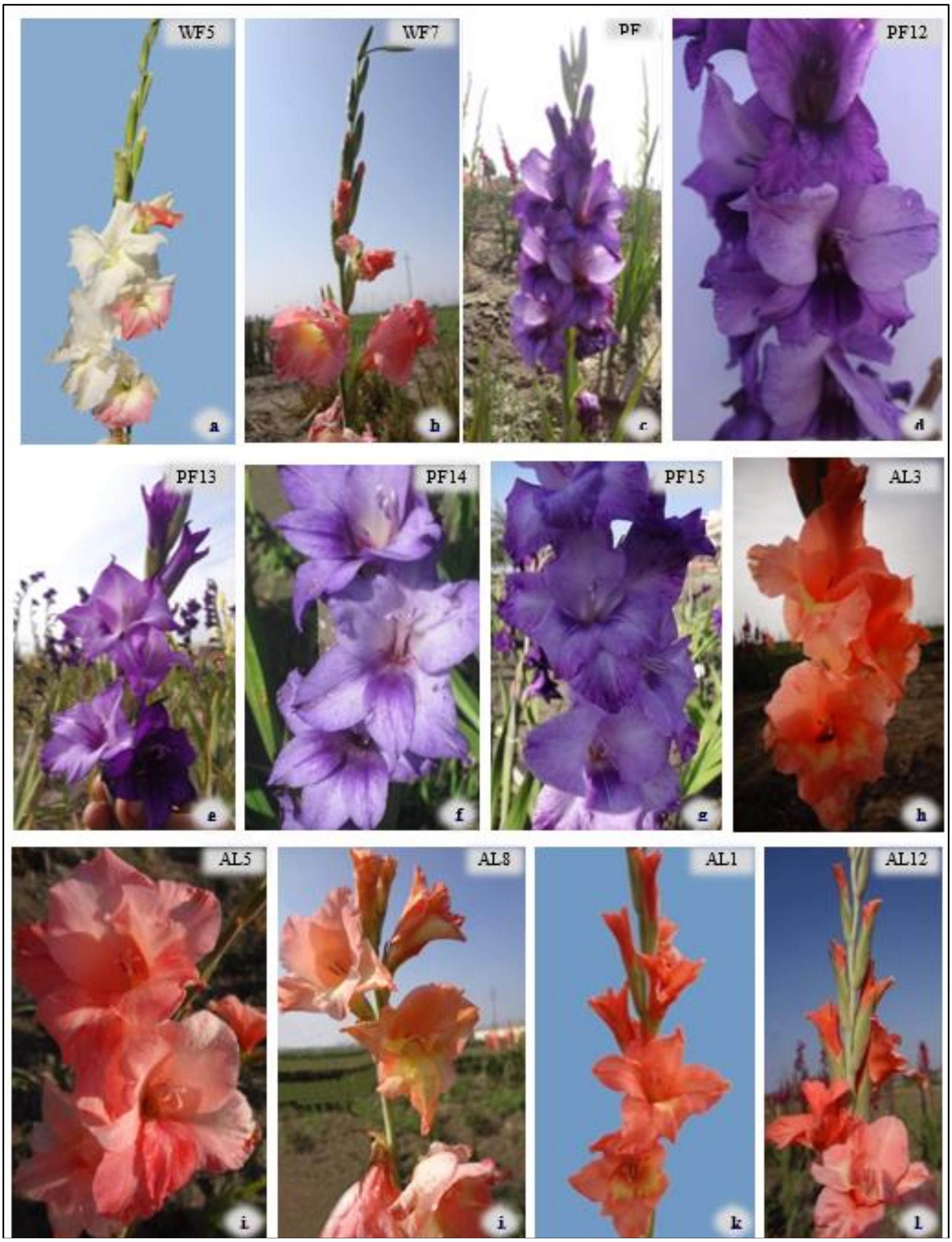

Plate 3: Flower colour mutants screened out from vM_3 generation of gamma irradiated corms of gladiolus varieties

Conclusion

The study showed that increase in gamma rays dose results in decreased survival of plants. Mutation frequency was high at 55 Gy dose in vM_1 generation and variants were in the form of chimeras whereas increased mutated sector size as well as solid mutants were recorded in vM_2 and vM_3 generation which shows that gladiolus is a promising species for mutation breeding if the right genotype is irradiated and selection is carried out in the second or third vegetative generation wherethe chances of getting wholly mutated plants are more. Total twelve mutants were isolated in vM_3 generation. Among eight varieties used, four varieties *viz*. Nathan red, White Friendship, Purple Flora and Algarve have good response for color variation where as wider spectrum of colour was recorded in variety Purple flora. As reduction in corm size occurred as a result of irradiation, the possibility of propagating gladiolus *in vitro* or *in vitro* mutagenesis might speed up the whole process of mutation induction, selection and subsequent propagation as well as useful mutants can be saved.

Acknowledgement

We acknowledge, Department of Science and Technology (DST), under the Ministry of Science and Technology, Government of India for providing fellowship under the "INSPIRE Programme" to carry out this research work. Our thanks are also due to Director Research and In charge Model floriculture Centre G.B.P.U A &, Pantnagr for providing all the facilities

References

1. Ahloowalia BS, Maluszynski M. Induced mutations-A new paradigm in plant breeding. Euphytica. 2001; 118:167-173
2. Broertjes C, Van Harten AM. Applied mutation breeding for vegetatively propagated crops. Elsevier, Amsterdam, 1988, 345.
3. Bhat TA, Khan AH, Parveen S. Spectrum and frequency of chlorophyll mutations induced by MMS, gamma rays and their combination in two varieties of Viciafaba L. Asian J Pl. Sci. 2005; 6(3):558-561.
4. Dhumal KN, Bolbhat SN. Induction of genetic variability with gamma radiation and its applications in improvement of horsegram. In:Adrovic, Feriz (Ed.), Gamma Radiation. In Tech Publisher, Croatia, 2012, 207-228.
5. Dhaduk BK. Induction of mutations in garden gladiolus (*Gladiolus L.*) by gamma rays. Ph.D. Thesis, IARI, New Delhi, 1992.
6. Esnault AM, Legue F, Chenal C. Ionizing radiation: advances in plant response. Environ. Exp. Bot. 2010; 68:231-237
7. Kumari K, Dhatt KK, Kapoor M. Induced mutagenesis in Chrysanthemum morifoliumvariety 'Otome Pink' through gamma irradiation. The Bioscan. 2013; 8(4):1489-1492.
8. Girija M, Dhanavel D, Gnanamurthy S. Gamma rays and EMS induced flower colour and seed mutants in cowpea (*Vigna unguiculata* L. Walp). Advances in Applied Science Research. 2013; 4(2):134-139.
9. Grotewold E. The genetics and biochemistry of floral pigments. Annu. Reviw. Plant Biology. 2006; 57:761-780
10. Rather ZA, Jhon AQ, Zargar GH. Effect of Co-60 Gamma rays on Dutch Iris-II. J Orn. Hort. New Series. 2002; 5(2):1-4.
11. Roychowdhury R, Tah J. Germination behaviors in M2 generation of Dianthus after chemical mutagenesis. Int. J Adv. Sci. Technol. Res. 2011; 1(2):448-454.
12. Sood M, Pathania NK. Spectrum and frequency of induced macro mutations in M2 generation of French bean (*Phaseolus vulgaris* L.). Veg. Sci. 2007; 34(1):14-17.