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Sheeba Belwal
Department of Horticulture, College of Agriculture, Govind Ballabh Pant University of Agriculture and Technology, Pantnagar, Uttarakhand, India

Ranjan Srivastava
Department of Horticulture, College of Agriculture, Govind Ballabh Pant University of Agriculture and Technology, Pantnagar, Uttarakhand, India

NK Singh
Department of Genetics and Plant Breeding, College of Agriculture, Govind Ballabh Pant University of Agriculture and Technology, Pantnagar, Uttarakhand, India

BD Bhuj
Department of Horticulture, College of Agriculture, Govind Ballabh Pant University of Agriculture and Technology, Pantnagar, Uttarakhand, India

Ajit Kumar
Department of Horticulture, College of Agriculture, Govind Ballabh Pant University of Agriculture and Technology, Pantnagar, Uttarakhand, India

Satish Chand
Department of Horticulture, College of Agriculture, Govind Ballabh Pant University of Agriculture and Technology, Pantnagar, Uttarakhand, India

Kanchan Karki
Uttarakhand Council of Biotechnology, Haldi, Pantnagar, Uttarakhand, India

Corresponding Author:
Sheeba Belwal
Department of Horticulture, College of Agriculture, Govind Ballabh Pant University of Agriculture and Technology, Pantnagar, Uttarakhand, India

Screening of mutants of gladiolus cultivar prince of orange in M1 and M2 generations

Sheeba Belwal, Ranjan Srivastava, NK Singh, BD Bhuj, Ajit Kumar, Satish Chand and Kanchan Karki

Abstract
An experiment was carried out to induce mutations, work out effective dose of physical and chemical mutagens and to isolate the desirable mutants and were screened in two consecutive growing seasons i.e. 2021-2022 and 2022-2023. The standard size corms of gladiolus var. Prince of Orange were treated with different doses of Co- 60 gamma irradiation source (4.0 to 5.5 Kr) and Ethyl methane sulfonate (EMS) (0.2 to 0.6% for 6 hours). Observations were recorded for vegetative and floral characters in each treatment to assess the effect of different mutagenic treatments. Four potential mutants characterizing variations based in floret colour (4.5 Kr and 5.5 Kr), plant height (4.0 Kr, 4.5Kr, 5.5Kr, 0.6%) and number of florets (0.6%) have been screened due to their desirable qualities.

Keywords: Gladiolus, gamma irradiation, chemical mutagen, vegetative and floral character

Introduction
In India, among the commercial flowers, gladiolus is one of the most important flowers because of its majestic spikes having attractive, elegant and delicate florets of various shades. This tender plant has a sequential opening of flowers that lasts long duration and possess good keeping quality as cut flowers [1]. It is being cultivated over an area of 11.66 thousand hectare with a production of 10.46 (loose flower) and 249.18 (cut value) thousand tone [2]. Gladiolus is rated fourth in worldwide trade, third in India's cut flower output and sixth in loose flower production [3]**.** Its current status makes gladiolus a very popular cut flower crop grown during summers in temperate to sub temperate areas and during winters in tropical to sub-tropical areas in India under open field conditions [4]. Also popularly called as "Queen of bulbous flower crops", gladiolus shows wide adaptability under different spectrum of agro climatic conditions which makes its availability possible throughout the year. Historically, the name gladiolus was initially coined by Pliny the Elder, deriving its basis from the Latin word *gladiolus*, meaning a sword as its foliage is sword-shaped. Because of this sword like leaves, it is popularly called as the 'sword lily' and also 'corn flag' in Europe. Gladiolus is an extremely heterozygous crop which possess a highly complex and dyanamic genetic constitution and is propagated through corms & cormels (vegetative propagation). As this crop is propagated vegetatively and is highly heterozygous, mutation breeding offers great opportunities to experiment and bring about desirable variations as the mutated part can be easily perpetuated by vegetative means without changing the surplus in vegetatively propagated crops [5]. Induced mutagenesis is well recognized as one of the important technologies for the development of new varieties through genetic manipulations. Mutation techniques, using physical and chemical mutagens, have successfully produced quite a large number of new promising varieties in different ornamental plants. The fact that the ever increasing demand of gladiolus in floriculture market calls for attention towards genetic improvements which can be made available in shorter durations cannot be neglected. To study such variations in the mutant population, morphological traits have long been used to estimate systematic relationships in crops and ornamentals since they are simple, fast and inexpensive to detect phenotypic changes if present. They are the oldest and most widely used markers and may still be considered suitable for identification of cultivars on the basis of various plant characters like plant shape, colour, size and form. Keeping this awareness in mind, a study was done to induce and isolate mutants based on some of the phenotypic characters that were different from those of control.

Materials and Methods

The present investigation was conducted at Model Floriculture Centre, Govind Ballabh Pant University of Agriculture and Technology, Pantnagar (Uttarakhand) in a randomized block design during 2021-2022 and 2022-2023. The experimental material adopted for the present investigation was *Gladiolus grandiflorus* var. Prince of Orange. The bavistin (0.2%) pretreated medium sized corms were subjected to different doses of physical mutagen and chemical mutagens. Mutations were induced in the corms through physical mutagenesis (Gamma irradiation) and chemical mutagenesis (EMS) based on Lethal dose (LD_{50}) values. The corms of gladiolus were irradiated separately with four doses of gamma rays viz., 4.0 kR, 4.5 kR, 5 kR and 5.5 kR. The gamma irradiation treatment of gladiolus corms of various cultivars were carried out at Gamma Chamber Facility of Radiation & Isotropic Tracers Laboratory (RITL), College of Basic Science and Humanities, GBPUA&T, Pantnagar, Uttarakhand. For chemical mutation, the corms were treated with aqueous solution of EMS i.e. 0.2%, 0.4% and 0.6% for 6 hours each, thoroughly washed under tap water for one hour and subjected to air drying before planting in the field. The crop was raised under uniform and standard cultural practices. Corms having uniform diameter ranging between 4-6 cm were selected for the experiment. Corms were planted at 30 cm row to row and 20 cm plant to plant distance. To keep field free from weeds during the experimental period the field was periodically inspected and weeding was done manually. Irrigation was done periodically by flooding method of irrigation. The last irrigation was applied two weeks prior to harvesting of corms and cormels from the experimental field. The harvested corms of M1 were used as base population in M2 generation. Variations cause due to mutagenic treatments were observed carefully by inspecting the variations in vegetative growth and flowering characteristics between treated and non-treated corms in vM1 and vM2 generations. Table 1 gives the treatment details used for the experiment.

Table 1: Detail of treatments used for physical and chemical mutations.

S. No.	Treatment	Combination
Gladiolus variety Prince of Orange (V_1)		
1.	T_1	Control (untreated)
2.	T_2	4.0 Kr Gamma radiation
3.	T_3	4.5 Kr Gamma radiation
4.	T_4	5.0 Kr Gamma radiation
5.	T_5	5.5 Kr Gamma radiation
6.	T_6	0.2% EMS
7.	T_7	0.4% EMS
8.	T_8	0.6% EMS

Observations were recorded in each treatment to assess the effect of different mutagenic treatments on growth, flowering and corm characters. The corms and cormels were lifted about 60 days after cutting of spikes. The infected and injured corms and cormels were removed. After that, corms and cormels were cleaned by removing the old mother corm and other leaf residues.

All the observations were subjected to statistical analysis using R studio software.

Results and Discussion

Various morphological parameters under study show significant results according to ANOVA analysis. The data in Table 2 shows that the maximum number of days taken for sprouting of gladiolus corms in M1 was recorded in T_8 (13.83) and minimum was recorded in T_1 (6.66) while in M2 (Table 3) maximum days were recorded in T_7 (12.83). As far as plant height was considered, it was found maximum in T_8 (109.66 cm) and minimum in T_2 (51.50 cm) in M1, while in M2 it was found maximum for T_7 (111.00 cm) and minimum for T_2 (51.63 cm). Plant height decreased with increased dose of gamma radiation which is in accordance with the reduction of plant height reported earlier after gamma irradiation in rose [6]. The maximum number of leaves 90 days after planting was found maximum in T_8 (9.44) in M1 and T_8 (9.33) in M2 which synchronizes with the findings of [7] who reported that in gladiolus the number of leaves increases at lower doses due to activation of physiological substances present in corms while higher doses retard cell division by arresting mitotic cell division and causing ill effects on auxins and leads to reduced growth characters in plant. The most number of days to spike initiation was recorded in T_8 (106.50 days) in both the generations and lowest was obtained in T_5 (85.00 days). It can be observed that the spike length was more for EMS treated mutants as compared to gamma ray induced mutants which might be due to inhibition of growth. In both the generations, the maximum spike length was found in T_7 (105.53 cm) and minimum was found in T_3 (50.389 cm). The results correspond to findings of [8] who irradiated three varieties of gladiolus with gamma rays and observed that irradiation treatments caused decrease in spike length and floret size. Various other explanations were also offered for reduced growth following mutagenic treatments such as, irradiation in M1 generations is primarily attributed to inhibition or delay in mitosis [9] disturbances in physiological process like auxin and DNA synthesis, reduced auxin synthesis and chromosomal aberrations [10].

Table 2: Effects of various mutagenic treatments on corms observed in M1 season (2021) based on morphological characters. DS (Days to sprouting), PH (Plant height), NL (No. of leaves/ plant), DSI (Days to spike initiation), SL (Spike Length), NF (Number of florets/ spike), DF (Diameter of 1st floret in cm) and YC (Yield of corms).

Parameters	DS	PH (cm)	NL	DSI	SL (cm)	NF	DF(cm)	YC (lacs/ha)
Treatments	M1	M1	M1	M1	M1	M1	M1	M1
T_1	6.66	86.83	6.44	84.83	75.33	11.33	6.16	1.62
T_2	7.83	51.50	7.51	86.83	48.5	8.66	5.13	1.33
T_3	8.16	56.83	8.44	87.16	50.1	6.66	5.26	1.66
T_4	8.50	57.50	9.22	85.66	45.33	5.66	4.20	1.62
T_5	11.16	51.83	10.44	85.33	46.33	4.66	4.06	1.66
T_6	11.66	101.66	8.44	94.83	96.4	15.18	6.26	2.36
T_7	13.33	114.00	7.22	101.33	105.53	18.66	7.13	1.92
T_8	13.83	109.66	9.44	106.50	104.37	21.66	8.03	2.36
Mean	10.1412	78.7262	8.393	91.558	71.486	11.558	5.778	1.816
SE(m)	0.459	0.687	0.138	0.410	0.074	0.274	0.102	0.043
CD at 5%	1.332	1.993	0.400	1.190	0.214	0.795	0.296	0.124

Table 3: Effects of various mutagenic treatments on corms observed in M2 season (2022) based on morphological characters. DS (Days to sprouting), PH (Plant height), NL (No. of leaves/ plant), DSI (Days to spike initiation), SL (Spike Length), NF (Number of florets/ spike), DF (Diameter of 1st floret in cm) and YC (Yield of corms).

Parameters	DS	PH (cm)	NL	DSI	SL (cm)	NF	DF(cm)	YC (lacs/ha)
Treatments	M2	M2	M2	M2	M2	M2	M2	M2
T_1	6.63	87.33	6.22	85.33	75.33	12.36	6.33	1.43
T_2	8.23	51.63	7.25	87.66	48.66	8.36	5.26	1.32
T_3	7.90	57.00	8.33	86.33	50.63	6.22	5.53	1.64
T_4	9.23	56.83	9.30	87.66	45.53	5.44	4.40	1.63
T_5	10.67	52.83	7.55	84.66	45.66	6.44	4.13	1.31
T_6	12.83	100.66	8.33	95.66	96.4	15.62	6.53	2.34
T_7	13.33	111.00	9.11	100.66	105.53	18.58	7.26	2.26
T_8	12.16	108.00	9.33	106.66	104.37	21.58	8.06	2.05
Mean	10.122	78.16	8.181	91.827	71.513	11.825	5.935	1.786
SE(m)	0.726	2.355	0.287	0.742	0.089	0.061	0.071	0.074
CD at 5%	2.106	0.299	0.099	2.153	0.258	0.17	0.207	0.215

The maximum number of florets/ spike was found in T_8 (21.66) and minimum was found in T_5 (4.66) in M1 and in M2 it was found maximum in T_8 (21.58) and minimum in T_4 (4.66). While the maximum 1st floret diameter was obtained in T_8 in both the generations and the minimum was found in T_5 (4.06 cm). The highest corm yield was obtained in T_6 and T_8 (2.367 lacs/ha) and least was found in T_5 (1.31 lacs/ha) in M2 generation. Improvement in size and number of corms in saffron due to application of gamma irradiation has also been noticed by [11].

Table 3: Phenotypic comparison of isolated mutants with control as shown in Fig.1.

Treatment	Mutant	Mutant Characters	Utility
T_1 Control	-	Florets: 11-12/spike, deep orange, 80-84 cm plant height.	Medium height, good for open for garden purpose.
T_2 (4.0 Kr)	PO 4.0	Florets:5-6/ spike, light orange, 50-52 cm plant height.	Dwarf stature, can be used as pot plant for indoor decoration, bed purpose and herbaceous border.
T_3 (4.5 Kr)	PO 4.5	Florets: 5-6/ spike, orange with white patches on florets, 51-54 cm plant height.	Dwarf stature, can be used as pot plant for indoor decoration and edging.
T_4 (5.0 Kr)	PO 5.5	Florets: 8-10/ spike, maroon, 56-58 cm plant height.	Dwarf stature, can be used as pot plant for indoor decoration and bouquet making.
T_8 (0.6%)	PO 0.6	Florets: 18-21/ spike, deep orange, 108-110 cm plant height.	Very tall plant, large number of florets, lodging resistant and good for hedging purposes.

As per the findings of the experiment, chemical mutagens show a general trend of improved quality parameters over control as compared to the physical mutagen treatments as most significant changes where obtained in EMS treated gladiolus corms. Gamma irradiated corms showed inverse relation with increase in dose for various parameters such as plant height, days to spike initiation, spike length, number of florets per spike and diameter of florets while EMS treated corms showed a liner relationship with increase in dose for these parameters. such as EMS act primarily on base pairs of the DNA molecule and yield a higher number of gene mutations because of which chemical mutagens are generally considered to be superior to physical mutagens for induction of mutation[12].A comparison based on phenotypic characters has been shown in Table 3 between the control and mutants obtained. Several authors have reported identification of superior mutants obtained from gamma irradiation and EMS treatments for various characters in flower crop [13] According to the observations obtained, it can be said that gamma ray doses upto 5.5 kR and Ethyl Methane sulphonate up to 0.6 % is beneficial for inducing mutations in order to obtain desirable qualitative and quantitative characters of existing population over control. The variations obtained during the experimentation due to mutagens showed some stable gene

mutation in two generations. Thus the various changes in the morphological framework of gladiolus can be achieved through mutation breeding technique. The induction of a wide spectrum of genetic variability with physical and chemical mutagens supplements the available spontaneous variability for the crop improvement.

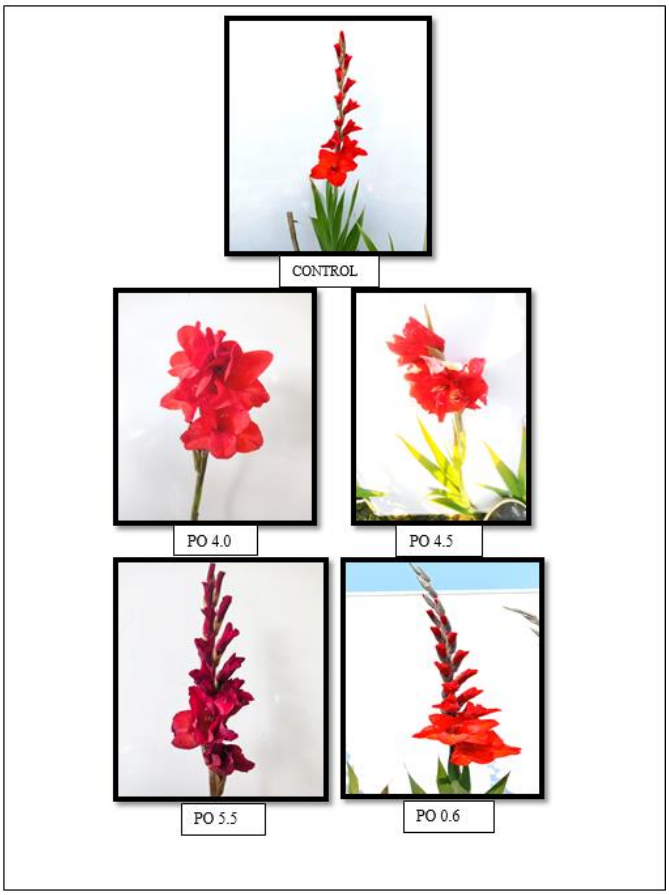

Fig 1: Stable mutants isolated from M1 and M2 generations of Prince of Orange.

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