Heliyon 10 (2024) e37387

Contents lists available at ScienceDirect

Heliyon

journal homepage: www.cell.com/heliyon

Research article

Determination of lethal and mutation induction doses of gamma rays for gladiolus (*Gladiolus grandifloras* Hort.) genotypes

Anand Singh Rawat [a,b], B.D. Bhuj [a], Ranjan Srivastava [a], Satish Chand [a], N. K. Singh [c], Yashpal Singh Bisht [b,d,*], Hemant Dasila [e,**], Rajendra Bhatt [f], Kahkashan Perveen [g], Najat A. Bukhari [g]

[a] *Department of Horticulture, College of Agriculture, Govind Ballabh Pant University of Agriculture and Technology, Pantnagar, Uttarakhand, India*
[b] *Department of Horticulture, Dr. Khem Singh Gill Akal College of Agriculture, Eternal University, Himachal Pradesh, India*
[c] *Department of Genetics and Plant Breeding, College of Agriculture, Govind Ballabh Pant University of Agriculture and Technology, Pantnagar, Uttarakhand, India*
[d] *Department of Vegetable Science, College of Agriculture, Govind Ballabh Pant University of Agriculture and Technology, Pantnagar, Uttarakhand, India*
[e] *Department of Microbiology, Akal College of Basic Science, Eternal University, Himachal Pradesh, India*
[f] *Department of Vegetable Science, College of Horticulture, VCSG Uttarakhand University of Horticulture and Forestry, Bharsar, Uttarakhand, India*
[g] *Department of Botany & Microbiology, College of Science, King Saud University, Riyadh, 11495, Saudi Arabia*

ARTICLE INFO

Keywords:
Cobalt 60
Survivability
Median blind dose
Gladiolus
Median lethal dose
Flower colour mutation frequency
Mutation spectrum

ABSTRACT

Gladiolus is a highly allogamous flower plant, but owing to the prolonged juvenile phase, asexual propagation is preferred, which acts as a barrier for the induction of natural genetic variability in gladiolus. Therefore, the induced mutagenesis could be utilized for the creation of desirable genotypes, without altering their basic agronomic features. An analysis of the optimum doses of γ radiation for the induction of fruitful mutations could be achieved in short period of time, compared with the conventional method of breeding. The objectives of this study were to perform radiosensitivity tests on various gladiolus genotypes using different doses of gamma rays and to determine the optimal dose of radiation dose for obtaining the greatest number of mutants. The present experiment was carried out during the winter-spring seasons, for the four consecutive years of 2017–18, 2018–19, 2019–20, and 2020–21. The seven genotypes of gladiolus were exposed to seven doses of gamma rays (^{60}Cobalt). Plants irradiated with radiation doses lower than 4.5 Kr (G_1) had greater plant survivability than the higher doses of gamma rays (≥5.0 Kr). The radiation of G_0 (0 Kr) result in highest plant survivability, while radiation dose of G_6 (6.5 Kr) resulted lowest survivability. LD_{25} and BD_{50} for all the genotypes were achieved except for V_5 and V_7, similarly the median lethal doses (LD_{50}) for V_3 and V_4 genotypes had been achieved. The highest flower blindness percent and percent abnormal plants were observed at G_5 and G_6 and between the 4.0 Kr (G_1) and 5.5 Kr (G_4) gamma ray doses, respectively. The flower colour mutation frequency was recorded highest in genotypes Tiger Flame at 5.0 Kr (V_7G_3), while the Flower colour mutation spectrum was identified between 4.0 Kr (G_1) to 5.5 Kr (G_4) in all the genotypes except for genotypes V_5 and V_7. For the generation of higher phenotypic variations, radiation dose between 4.0 Kr (G_1) and 5.5 Kr (G_4) were found the most prominent. Specifically

* Corresponding author. Department of Horticulture, Dr. Khem Singh Gill Akal College of Agriculture, Eternal University, Himachal Pradesh, India.
** Corresponding author. Department of Microbiology, Akal College of Basic Science, Eternal University, Himachal Pradesh, India
E-mail addresses: Yashpal.ktw@gmail.com (Y.S. Bisht), drhemantmicro@eternaluniversity.edu.in (H. Dasila).

https://doi.org/10.1016/j.heliyon.2024.e37387
Received 16 May 2024; Received in revised form 25 August 2024; Accepted 2 September 2024
Available online 4 September 2024

the gamma rays radiation dose of 5.5 Kr (G_4) resulted in the highest flower colour mutation frequency. These isolated mutant lines will broaden the gladiolus gene pool and support future gladiolus breeding experiments.

1. Introduction

Mutation is one of many beneficial ways to identify the of nature and behavior of a certain gene, which eventually broadens the genetic base of plant species and aids in the development of new gene pools or genotypes [1]. Aside from disrupting the link between some specified features, mutation can induce diversity in both quantitative and qualitative traits in the aimed population at each recognized as well as unrecognized DNA region [2]. It can be induced to create direct mutations or used in hybridization to improve output and establish favorable agronomic traits while lowering viability to the greatest extent feasible [3]. Induced mutation has significant potential and has been employed as an adjunct technique in plant genetic improvement [4]. Mutation breeding has made a significant involvement in the production of high yielding genotypes across the globe in the last five decades. Because of the extensive use of induced mutations, 3222 variations of over 170 plant species have been developed through plant breeding in over 60 countries [5]. Plant breeders are now using this strategy much more efficiently than ever before, because to a novel approach in mutant breeding that changes away from conventional mutation research and towards modern reverse genetics without the introduction of foreign genes, which may or may not be as sustainable as the natural form of respective plants. Advantageous mutations are alterations in genotypic structure that improve plant species heterogeneity and encourage adaptability to multiple selection environments [6]. This can be caused by physical mutagens such as X-rays and gamma rays (ionizing radiation), ultraviolet rays (nonionizing radiation), and protons, neutrons, alpha and beta particles (corpuscular radiation) [6–8].

The majority of mutant plant genotypes documented in the Collaborative FAO/IAEA mutant variety database were generated through gamma rays [9]. Gamma rays are chosen for inducing mutations due to their high energy and deep penetration capabilities, which result in a higher mutation rate. This makes them highly effective for generating genetic variations, thereby aiding in breeding and research efforts to develop improved plant traits. This can also be explained by ion formation from gamma irradiation penetrates tissues and influences mutation. These ions generate chemical reactions which damage the chromosomes and DNA. As a result of this damage, plant genomes are altered. Fast neutrons produce severe deletions, chromosomal loss, and translocations, all of which can often be lethal [10]. The most significant element influencing the biological consequences of ionizing radiation is the quantity of energy received by the biological system. Gladiolus is one of the plant species for which the impact and application of gamma rays for crop development have been thoroughly documented [11]. In contrast with the technique utilized to achieve genetically modified organisms (GMOs), the generation of new varieties by gamma radiation is random, but they can be detected and chosen rapidly speeding up the establishment and release process [6]. The International Atomic Energy Agency's (IAEA) database of mutant cultivars shows that 1703 plants of agronomic significance with mutations caused by gamma radiation were identified between 1960 and 2020. A bicolour and mold resistance are two benefits of the Rose mutant (Pink Hat), which was certified in 1960 and was created by radiating terminal buds. Mutants of *Bougainvillea* spp. and *Solanum lycopersicum* L. have recently been registered [6].

Determining the optimum radiation dosage, or the dose that eliminates 50 % and 25 % of the population (lethal dose, LD_{50} and LD_{25}), is requisite for the effective induction of mutagenesis by gamma radiation. Additionally, it needs factors like survival, mass, or the quantity of specimens that have sprouted, among others; alternatively, both dosages rely on the plant tissue, development stage, and moisture content, among other factors [12,13]. By influencing growth promoters and ultimately causing tissue damage, high radiation doses can cause radio inhibition [14]. In addition to tissue damage, it can also result in plant tissues becoming malformed and losing their ability to regenerate [15]. The flower blindness in gladiolus crop can be defined, as the plant which are unable to produce flower due extreme short day condition or low temperature. In gladiolus, treating corms with gamma rays can result in some plants being unable to produce flowers. This occurs due to disruptions in the biosynthesis of plant hormones responsible for flower development. These non-flowering plants are referred to as blind plants. The radiation dose at which plants fail to produce flowers is termed the blind dose. Radiation dose that causes flower blindness in half of the population (median blind dose, BD_{50}). According to Ref. [14], the words "radio-sensitivity test" and "optimum dose analysis in plant mutagenesis" refer to the relative degree of observable effects of radiation exposure on irradiated samples. Determining the somatic mutation, chromosomal breakage, mortality, or growth inhibition of large-scale induced mutations is a prerequisite. Variations occur in the chemical, physical, and biological variables that can alter the impact of radiation on plants. Tshilenge-Lukanda [16] stated that the optimal doses of mutation induction might be established through calculating seed germination percentages, survival percentages of seedlings, lengths of hypocotyl, and epicotyl.

Generally, mutagen doses which caused 50 percent lethality (LD_{50}) among the M_1 population may be suitable due to its ability to generate a higher range of mutations [17]. The objective of induction of mutation is to create genotypic and phenotypic variations, which are important for the selection of plants with desirable characteristics. Optimum potential of producing viable and useful mutants for genetic improvement of plant, the utilization of acute and chronic mutagenesis procedures might be obtained at higher doses where half of the radiated samples died [13]. LD_{50}, LD_{25}, and BD_{50} are typically determined based on the theory that lower gamma-irradiation levels can have the lower effect on the plant genome, potentially causing morphological changes; on the other hand, increased doses of gamma rays may have multiple effects on the entire genome, potentially resulting in unfavorable mutations [13,18].

Replication mutations can occur as base substitutions, base additions, or base deletions. When discussing mutations, it's important to differentiate between mutation frequency and mutation rate [19]. Mutation frequency is defined as the number of mutations or

variations detected at a particular mutagen dose per population of cells, organisms, gametes, plants, or plant parts during a single mutant generation [20]. Mutation frequency is typically expressed as a proportion of mutants from radiated corms (M_0), surviving plants in the first mutant generation (M_1), or mutated plants in subsequent generations [21]. The mutations can be observed mostly in the forms of chimeras, variegations, and colour blends in flower spike of gladiolus and may also found stable in subsequent generations. Gamma ray doses can sometimes adversely affect plants, leading to abnormalities such as fusion of tepals and sepals, multiplication of tepals, doubling of stamens and carpel, shortening of spikes, etc. It is apparent that the radio-sensitivity to gamma radiation in numerous ornamental plants is essentially a genotype-dependent process. While some of the types have been identified to be quite sensitive to mutagens, others were found to be extremely sensitive. It is true that distinct mechanisms for pigment production determine the hue of flowers. Varieties with varying hue variations respond differently. The incidence of flower colour mutation varied with gamma irradiation dosage and variety. Similarly, the mutation spectrum also varied with the dosages of gamma radiation.

2. Material and methods

2.1. Study site and plant material

The current filed experiment was carried out in the Model Floriculture Centre of G. B. Pant University of Agriculture and Technology, Pantnagar, Uttarakhand (India) falls under in lowland region of Himalayas, during the winter-spring seasons for the four consecutive years 2017–18, 2018–19, 2019–20, and 2020–21. The experiment site has subtropical and humid climate conditions with an elevation of 243.84 m above mean sea level in the foothills of the Himalayas at 29.01 °N latitude and 79.48 °E longitude. The seven genotypes were selected for the current investigations for their unique for colour, texture, yield and quality. The phenotypic and genotypic details of the genotypes are given in Table 1. The genotypes used in the present study were sourced from the Indian Institute of Agriculture Research, New Delhi (India) and Banaras Hindu University (BHU), Varanasi, Uttar Pradesh (India).

2.2. Irradiation treatment and experiment design

Uniform and standard sized corms of gladiolus from seven cultivars namely, Nova Lux (V_1), Praha (V_2), Black Star (V_3), Nathan Red (V_4), Candyman (V_5), Punjab Dawn (V_6) and Tiger Flame (V_7) were exposed to seven doses of gamma radiation viz., 4.0 Kr (G_1), 4.5 Kr (G_2), 5.0 Kr (G_3), 5.5 Kr (G_4), 6.0 Kr (G_5), 6.5 Kr (G_6) and 0.0 Kr (control) (G_7). Cobalt 60 (^{60}Co) based gamma chamber GC-5000 was used for the acute gamma irradiation treatment available in the Radiations and Isotopic Tracers Laboratory (R.I.T.L.), CBSH, G.B. Pant University of Agriculture and Technology, Uttarakhand (India). Irradiated corms than planted in experiment site for further evaluation.

The total forty nine treatment combination of genotypes, gamma rays and their interaction were subjected for the study in Randomized Block Design with three replications. In each replication, there were sixty corms or plants, and a total of 147 replications of all treatment combinations. The intercultural operations such as weeding, staking and irrigation were done as per need. The radiated genotypes along with one control (non-irradiated corms) were the studied for the morphological characteristics *viz.,* plant survivability, mortality, percent abnormal plant, percent blind plants, Mutation colour frequency and mutation spectrum.

2.2.1. Post irradiation plant culture and method of evaluation of phenotypic data

After the successful irradiation of gladiolus corms with the above mentioned doses of gamma rays, planting of corms was done in the experiment field. Uttarakhand's lowland area is renowned for having a humid subtropical climate. Winter temperatures often range from 0 °C to 9 °C, while summer temperatures typically range from 30 °C to 43 °C. The area has hot, dry summers and chilly winters, along with high rainfall of up to 1400 mm yearly. The Monsoon season typically lasts from mid-June until the end of September. Typically, frost begins around the final week of December and continues through the end of January. Between mid-June and the end of February, relative humidity normally ranges from 80 to 90 %; during the first week of May, it drops to 50 % and remains there until mid-June. The experiment was conducted under the open field condition. The corms were planted in the raised bed at a spacing of 30 cm plant to plant and 30 cm row to row, after dipping corms in the systemic fungicide for 30 min. Treating gladiolus corms with a systemic fungicide protects the plants from contamination and infection by soil-borne fungal diseases. Additionally, the application of systemic fungicides does not have any genotoxic effects on gladiolus. The phenotypic variations were assisted by visual screening of the irradiated plants for their novel flower colour. After visual screening, the mutants were subjected to a colour

Table 1
Detailed list of genotypes and doses of gamma radiation.

S/N	Genotype	Notation	RHS[a] Colour	S/N	Doses	Notation	Source
1	Nova Lux	V_1	Yellow Group 4C	1	4.0 Kr	G_1	^{60}Co
2	Praha	V_2	Red Group 40A	2	4.5 Kr	G_2	^{60}Co
3	Black Star	V_3	Red Purple Group 59A and 59 B	3	5.0 Kr	G_3	^{60}Co
4	Nathan Red	V_4	Red Purple Group 72A	4	5.5 Kr	G_4	^{60}Co
5	Candyman	V_5	Purple Group C	5	6.0 Kr	G_5	^{60}Co
6	Punjab Dawn	V_6	Red Group 49A	6	6.5 Kr	G_6	^{60}Co
7	Tiger Flame	V_7	Orange Group 29A and 27B	7	0.0 Kr	G_0	Control

[a] Royal Horticulture Society.

conformity test using the Royal Horticulture Society's colour chart. The isolated desirable mutants were planted and screened for their colour stability in subsequent generations.

2.3. Data collection

The observations for the plant survivability (%), Lethal dose (LD_{25} and LD_{50}), Blind dose (BD_{50}), Mortality (%), Percent abnormal plant, Percent blind plants, Mutation colour frequency and Mutation spectrum were recorded during the course of investigation by following the methods described by FAO/IAEA [6,8,22].

2.3.1. Plant survival percentage (%)

The corm sprouting or survivability percent was counted after two weeks of planting in experiment field. The survivability percentage was calculated by the formula (Equation 1).

$$Plant\ Survival\ percentage(\%) = \frac{Numbers\ of\ corm\ sprouted}{number\ of\ corms\ treated} \times 100 \quad (1)$$

2.3.2. Radio sensitivity test

Based on plant survival and mortality percentages (%) following gamma radiation treatment, the radio sensitivity test was assessed. Lethal dose percentages (LD_{25} and LD_{50}) was evaluated after two weeks of sprouting in open field condition.

By fitting the straight-line equation for the model, the mean lethal dose (LD_{25} and LD_{50}) was plotted using a simple linear regression approach (Equation (2)). Graph of absorbed dosage of gamma rays versus plant survival percentage.

$$y = mx + c \quad (2)$$

where y is the outcome factor (the percentage of plants that survive), x is the independent variable (gamma ray dosages), and m and c are the slope and constant, respectively.

2.3.3. Blind dose (BD_{50})

The mean blind dose (BD_{50}) was determined by plotting the straight-line equation for the model, a basic linear regression model graph of absorbed dosage of gamma radiation against flower blindness percentage (Equation (3)).

$$y = mx + c \quad (3)$$

where y is the outcome factor (the percentage of plants that survive), x is the independent variable (gamma ray dosages), and m and c are the slope and constant, respectively.

The data were also observed and collected on plant survivability percentage, mortality (%), percent blind plants, percent abnormal plants, mutation frequency (%) and colour mutation frequency (%) for each genotype, gamma irradiation treatment and their interactions as follows.

2.3.4. Percent blind plants (%)

The percent blind plants was counted after completion of flowering period. The percent blind plants was calculated by using following formula (Equation (4)).

$$Percent\ blind\ plants\ (\%) = \frac{Numbers\ of\ blind\ plants}{Total\ number\ of\ Plants} \times 100 \quad (4)$$

2.3.5. Percent abnormal plants (%)

The percent abnormal plants was counted after 30 days of germination. The percent abnormal plants was calculated by using following formula (Equation (5)).

$$Percent\ Abnormal\ plants\ (\%) = \frac{Numbers\ of\ Abnormal\ plants}{Total\ number\ of\ Plants} \times 100 \quad (5)$$

2.3.6. Flower colour mutation frequency (%)

The flower colour mutation frequency data from four consecutive generations were combined and computed using the following method (Equation (6)).

$$Flower\ Colour\ Mutation\ Frequency\ (\%) = \frac{Numbers\ of\ plants\ having\ Colour\ variation}{Total\ number\ of\ irradiated\ Plants} \times 100 \quad (6)$$

The data for the mutation spectrum were observed by comparing the mutant plant flower colour or variation with their parent plant or flowers from the control plants by using Royal Horticulture Colour (RHS) chart. The variation were recorded in the form of colour code and pictures.

2.4. Data analysis

The present study utilized analysis of variance for a completely randomised design that included two factors, namely varieties and Gamma rays, and three replications [23]. This allowed for a statistical analysis of the data acquired for various plant metrics. In accordance with [24], the square root transformation of the percentage data was used to achieve the model assumptions for the analysis of variance. IBM SPSS statistics, version 26, were used as a statistical tool for the analysis of data regarding the plant survival percentage (%), percent blind plant (%), percent abnormal plants (%) and mutation frequency. The Probit analysis was performed to estimate the optimal gamma radiation dosage in the dosage-response analysis to evaluate radiation tolerance of the crop species and to indicate a target dose for large-scale experimentation. The LD_{25} and LD_{50} values were determined from plant survival percentage and mortality (%) data of plants using probit analysis, a kind of regression that uses Microsoft Excel to examine binomial response variables and converts the sigmoid dose-response curve into a straight line, as described by Refs. [25,26]. In case of flower colour mutation frequency and spectrum, the data collected from vM_1, vM_2, vM_3 and vM_4 generations was pooled and analyzed for F-test. Only frequency distribution and the variance around the average of irradiated and non-irradiated plants (control) with novel traits were performed because data have been collected from particular plants, which were not replicated.

3. Results

3.1. Plant survival percentage

Plant survival percent (%) of different genotypes of gladiolus on different doses of gamma radiations were calculate by the comparing the sprouting or survival percent of untreated corms. Among the treatments a significant reduction ($P < 0.01$) in sprouting percent of corms were observed over the control, which resulted the overall reduction of plant survival percentage (Table 2). Among the genotypes, highest plant survival percentage was observed in V_5 (94.04 %) while the lowest was recorded in genotype V_4 (78.82 %). Among the gamma radiation doses, the highest plant survival percentage was recorded in non-irradiated corms (G_0) (98.87 %) whereas, lowest survivability was found at G_6 (65.80 %) dose (Table 3). Data for interactions of genotypes and gamma ray doses indicate that, the highest plant survival percentage was recorded in V_5G_0 (99.46 %) followed by V_4G_0 (99.34 %) and V_2G_0 (99.30 %) whereas, lowest plant survivability was found in V_4G_6 (34.64 %) followed by V_4G_5 (45.78 %) (Table 4).

3.2. Lethal dose (LD_{25} and LD_{50})

Based on mortality data and probit analysis, a liner increase in plant mortality was observed with the increase in gamma ray doses under field condition (Fig. 1). The lethal dose (LD_{25}) for the genotypes ranges from 5.48 to 10.10 Kr, similarly median lethal dose (LD_{50}) ranges from 5.90 to 22.15 Kr, which indicating the targeted dose for getting the optimum numbers of mutations from the genotypes. More specifically, LD_{25s} for V_1, V_2, V_3, V_4, V_5, V_6 and V_7 were determined at 6.19, 5.98, 5.48, 5.72, 10.11, 6.10 and 7.34 Kr, respectively (Fig. 1A–G). Whereas median lethal dose (LD_{50s}) for the genotypes V_1, V_2, V_3, V_4, V_5, V_6 and V_7 were recorded at 8.95, 8.12, 6.31, 5.90, 22.15, 8.12 and 14.69 Kr, respectively. The R^2 values and equations for different genotypes are depicted in (Fig. 1 (A–G).

3.3. Blind dose (BD_{50})

Blind dose (BD_{50}) could be the significant measurement of inhibitory effect of gamma rays on flower bud initiation and other important physiological, chemical and morphological events in plant system. The careful study and analysis of flower blindness data of gladiolus plant can be unfold the accurate prediction of flower blindness per cent or events in plants at specific gamma ray dosses. A significant increment were observed in flower blindness in all the genotypes with the increase in gamma ray doses. The R^2 values and straight line equation for different genotypes are given in (Fig. 2A–G). Based on flower blindness percent (%) data and probit analysis, the blind dose (BD_{50}) for genotypes V_1, V_2, V_3, V_4, V_5, V_6 and V_7 were calculated to be 6.43, 6.31, 3.29, 2.95, 13.0, 5.40 and 6.95 Kr respectively (Fig. 2A–G).

Table 2
Mean sum of square values and significance test for the survival percentage (%), percent blind plants (%), percent abnormal plants (%) and flower mutation frequency (%) of genotypes, gamma ray doses and their interactions.

Source of Variation	Df	Mean Sum of Square			
		Survival Percentage (%)	Percent blind Plants (%)	Percent Abnormal Plants (%)	Flower Colour Mutation Frequency (%)
Genotypes	6	707.84**	3,647.13*	149.56*	50.06*
Gamma Ray Doses	6	2905.43**	6,328.83**	240.49*	12.17*
Genotypes × Gamma Ray Doses	36	252.21**	431.41*	45.32	8.79*
Error	96	1.25	9.15	16.05	0.003

Where, df degree of freedom, ** Significant at $p \leq 0.01$, * Significant at $p \leq 0.05$.

Table 3
Mean survival percentage (%), percent blind plants (%) and percent abnormal plants (%) of seven gladiolus genotypes and seven gamma ray doses.

Treatments	Survival percentage (%)	Percent blind plants (%)	Percent abnormal plants (%)	Flower colour mutation frequency (%)
Genotypes (Factor A)				
V_1	85.24	37.72	4.49	0.33
V_2	85.50	32.31	5.11	0.67
V_3	79.32	60.02	1.10	0.23
V_4	78.82	62.69	3.20	0.00
V_5	94.05	29.74	3.71	3.88
V_6	87.95	43.84	9.83	1.44
V_7	92.06	36.17	4.29	3.17
Gamma Ray Doses (Factor B)				
G_1	92.59	36.35	7.59	1.15
G_2	92.26	50.23	6.23	1.69
G_3	91.20	40.65	9.07	2.00
G_4	88.16	51.98	5.13	2.20
G_5	74.08	57.17	2.41	0.90
G_6	65.80	57.83	1.29	1.76
G_0	98.87	8.29	0.00	0.00
$CD_{(0.05)}$	0.687	1.856	2.458	0.033
SE(D) ±	0.345	0.933	1.236	0.017
SE(M) ±	0.244	0.66	0.874	0.012

3.4. Percent blind plants (%)

Pooled data showed significant effect of genotypes, gamma irradiation doses and their interactions on per cent blind plants (Table 2) The pooled data regarding effect of genotypes revealed that, percentage of blind plants was found highest in genotype V_4 (62.69 %) whereas it was found least in V_5 (29.74 %). Among the gamma radiation doses, highest percentage (57.83 %) of blind plants was recorded in G_6 closely followed by G_5 (57.17 %) dose of gamma radiation whereas, lowest per cent blind plants (8.29 %) was found in untreated corms (G_0) (Table 3). The interaction effect of irradiation doses and genotypes on blind plants percentage showed that, it was highest in V_4G_6 (100 %), whereas least percentage of blind plants was recorded in V_4G_0 (4.76 %) (Table 4).

3.5. Percent abnormal plants (%)

The pooled data for per cent abnormal plants has been presented in Table 4. Among the genotypes, lowest percentage of abnormal plant was observed in genotype V_3 (1.10 %), while the highest percentage of abnormal plants was found in V_6 (9.83 %) (Table 3). Among the gamma ray doses, the minimum per cent abnormal plant was recorded in G_6 (1.29 %) dose whereas, maximum per cent abnormal plant was found at V_3 (9.07 %). Data for interaction of genotypes and gamma radiation doses exhibited non-significant differences (Table 2) for per cent abnormal plants, the minimum per cent abnormal plant was recorded in V_6G_2 (21.43 %), while minimum (0.83 %) were recorded in V_3G_3 (Table 4).

3.6. Flower colour mutation frequency and spectrum

The pooled data of four consecutive generations showed that, genotype V_5 (3.88 %) had the maximum flower colour mutation frequency followed by V_7 (3.17 %) dose of gamma rays, while lowest was observed V_4 (0.00 %) followed by V_3 (0.23 %) (Table 3; Fig. 3). Among the gamma ray doses, the highest flower colour mutation frequency was recorded in G_4 (2.20 %) dose followed by G_3 (2.00 %) whereas, lowest was recorded at G_0 (0.00 %). The interactive effect showed that, the highest flower colour mutation frequency was found in V_7G_3 (9.05 %) followed by V_5G_6 (7.66 %), whereas second lowest observation was recorded in V_1G_4 (0.44 %) (Table 4). The flower colour mutation spectrum (FCMS) for all the genotypes under investigation were observed between 4.0 and 6.5 Kr except for V_4. The FCMS for V_1 (4.0–5.5 Kr), V_2 (4.5–5.5 Kr), V_3 (4.5 Kr), V_5 (4.0–6.5 Kr), V_6 (4.0–6.5 Kr) and V_7 (4.5–6.5 Kr) (Table 4; Fig. 3).

4. Discussion

4.1. Plant survival percentage (%)

In the current investigation, a very significant interactions were identified between the genotypes and dosages of gamma radiations for the plant survival percentage (%) (Tables 3 and 4). The sudden reduction in plant survival percentage and mortality may result from several factors, including the detrimental effects of cytochrome oxidase concentration and reduction in respiration [27,28], drastic distortion of the actively dividing phase [29]; molecular destruction of cell constituents; altered enzyme activity or block in cellular DNA formation causing the plant growth to cease or slow down [30,31]; suppression of auxin and gibberellins syntheses [32,33]; disruption in the synthesis of enzyme activity and acceleration in the degradation of existing enzymes [34]; nature and extent of chromosomal damage [35]. These physiological factor might be involved in the low plant survivability of plants over the generations

Table 4
Mean survival percentage (%), percent blind plants (%), percent abnormal plants (%) and flower mutation frequency (%) of forty nine treatment combinations of genotypes and gamma ray doses.

Treatments (Factor A × Factor B)	Survival Percentage (%)	Percent Blind Plants (%)	Percent Abnormal Plants (%)	Flower Colour Mutation Frequency (%)
V_1G_1	88.23	32.96	7.64	1.11
V_1G_2	90.18	47.02	8.55	0
V_1G_3	93.38	39.88	11.54	0.73
V_1G_4	87.36	37.91	3.69	0.44
V_1G_5	74.57	47.39	0.00	0
V_1G_6	64.33	53.14	0.00	0
V_1G_0	98.62	5.76	0.00	0
V_2G_1	90.23	10.77	7.49	0
V_2G_2	93.23	45.72	3.55	0.66
V_2G_3	94.62	31.26	15.44	1.57
V_2G_4	86.80	25.42	9.28	2.45
V_2G_5	73.71	48.57	0.00	0
V_2G_6	60.63	55.17	0.00	0
V_2G_0	99.30	9.29	0.00	0
V_3G_1	86.38	70.19	6.85	0
V_3G_2	87.45	76.06	0.00	1.59
V_3G_3	89.15	39.60	0.83	0
V_3G_4	81.76	86.40	0.00	0
V_3G_5	64.34	75.38	0.00	0
V_3G_6	47.78	64.71	0.00	0
V_3G_0	98.40	7.77	0.00	0
V_4G_1	93.56	41.39	6.94	0
V_4G_2	93.17	68.21	2.19	0
V_4G_3	93.45	55.62	13.26	0
V_4G_4	91.81	82.15	0.00	0
V_4G_5	45.78	86.67	0.00	0
V_4G_6	34.64	100.00	0.00	0
V_4G_0	99.34	4.76	0.00	0
V_5G_1	95.79	33.68	3.32	4.13
V_5G_2	92.69	38.10	3.88	5.3
V_5G_3	94.17	31.57	3.82	1.34
V_5G_4	96.77	38.31	9.09	7.18
V_5G_5	89.55	31.56	1.67	1.57
V_5G_6	89.93	25.85	4.17	7.66
V_5G_0	99.46	9.10	0.00	0
V_6G_1	97.31	39.94	12.42	2.82
V_6G_2	94.90	48.46	21.43	2.46
V_6G_3	89.27	52.17	5.56	1.32
V_6G_4	81.34	47.50	11.42	2.15
V_6G_5	75.31	51.96	13.14	0
V_6G_6	78.36	53.77	4.86	1.33
V_6G_0	99.18	13.11	0.00	0
V_7G_1	96.63	25.51	8.44	0
V_7G_2	94.17	28.03	4.01	1.85
V_7G_3	84.41	34.44	13.05	9.05
V_7G_4	91.29	46.17	2.44	3.17
V_7G_5	95.25	58.66	2.08	4.76
V_7G_6	84.92	52.18	0.00	3.33
V_7G_0	97.77	8.20	0.00	0
$CD_{(0.05)}$	1.818	4.909	6.503	0.087
SE(D) ±	0.914	2.469	3.271	0.044
SE(M) ±	0.646	1.746	2.313	0.031

as evident by the earlier studies. According to Ref. [36], a high mutagen dosage rate results in an increased proportion of alterations such as chromosomal abnormalities, mortality, damage, and sterility. The current investigation demonstrated that greater doses of gamma rays (≥5.0 Kr) had a more substantial impact on plant survival than the lower doses (≤4.5 Kr). Optimisation of mutation frequency is critical and it must be empirically estimated; if it is excessively low, plenty of plants will be required to locate mutations in the targeted gene; if it is overly high, plant survivability, sterility, abnormalities, and mortality will likely to be issues, especially when unfavorable. Plant sterility, mortality in the first and second generation, apical meristem growth halt, tumorigenesis, pollen non-viability were also observed by Ref. [37] in gladiolus and chilli pepper [38]. As noted by Refs. [39,40], the observed variations in biological traits imply that the determined optimal dosages of single gamma radiation may result in beneficial mutants.

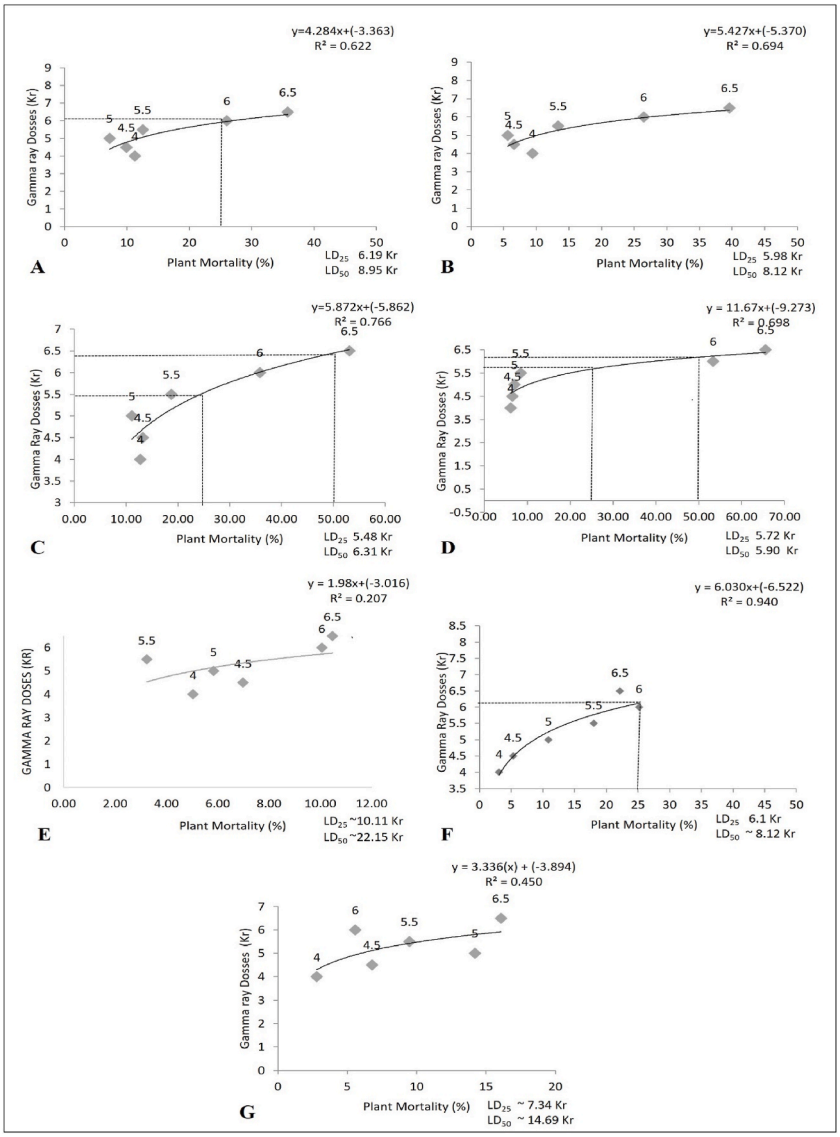

Fig. 1. Patters (A–G). Determination of Lethal Dose (LD_{25} and LD_{50}) of gamma ray doses on different genotypes of gladiolus using probit analysis (A) Nova Lux, (B) Praha, (C) Black Star, (D) Nathan Red, (E) Candyman, (F) Punjab Dawn and (G) Tiger Flame.

4.2. Lethal dose (LD_{25} and LD_{50})

Over the generations, a highly significant interaction has been observed for plant mortality (%) between the genotypes and gamma ray dosses. The LD_{25s} and LD_{50s} were calculated on the basis of plant mortality and survivability in four consecutive generations (vM_1, vM_2, vM_3 and vM_4). LD_{25s} and LD_{50s} values for different gladiolus genotypes V_1, V_2, V_3, V_4, V_5, V_6 and V_7 was found at 6.19 and 8.95 Kr, 5.98 and 8.12 Kr, 5.48 and 6.31 Kr, 5.72 and 5.90 Kr, 10.11 and 22.15 Kr, 6.10 and 8.12 Kr and 7.34 and 14.69 Kr, respectively (Fig. 1A–G). The present finding indicates that we had achieved the specific doses of gamma radiations to obtain the LD_{25s} for all the genotypes except V_5 and V_7 (Fig. 1A–G). The median lethal doses (LD_{50}) for V_3 and V_4 genotypes had been achieved under present study but for the rest genotypes median lethal doses were not achieved yet (Fig. 1C–D). Therefore, irradiation with higher doses of gamma rays ($\geq$6.5 Kr) may needed to obtain median lethal doses for the genotypes V_1, V_2, V_5, V_6 and V_7. The lethal dose (LD_{50}) is believed to be have a crucial factor to assess the level of radio-sensitivity in plants [41]. The researchers emphasized the need of utilizing the optimal dosage rate to achieve beneficial and healthy plant populations that can be brought to maturity. When Singh and Bala [34] exposed Chrysanthemum flowers to gamma radiation dose rates ranging from 0 Gy to 30 Gy, they discovered that LD_{50} values calculated using plant survival rate were more appropriate for large-scale mutagenesis than those derived using sprouting percentage data. This outcome confirms the observations in gladiolus [42] for several genotypes between 10 Kr and 15 Kr, chrysanthemum [33,43], in which they observed median lethal dose at 2.5 Kr and 3.0 Kr dose of gamma rays, respectively.

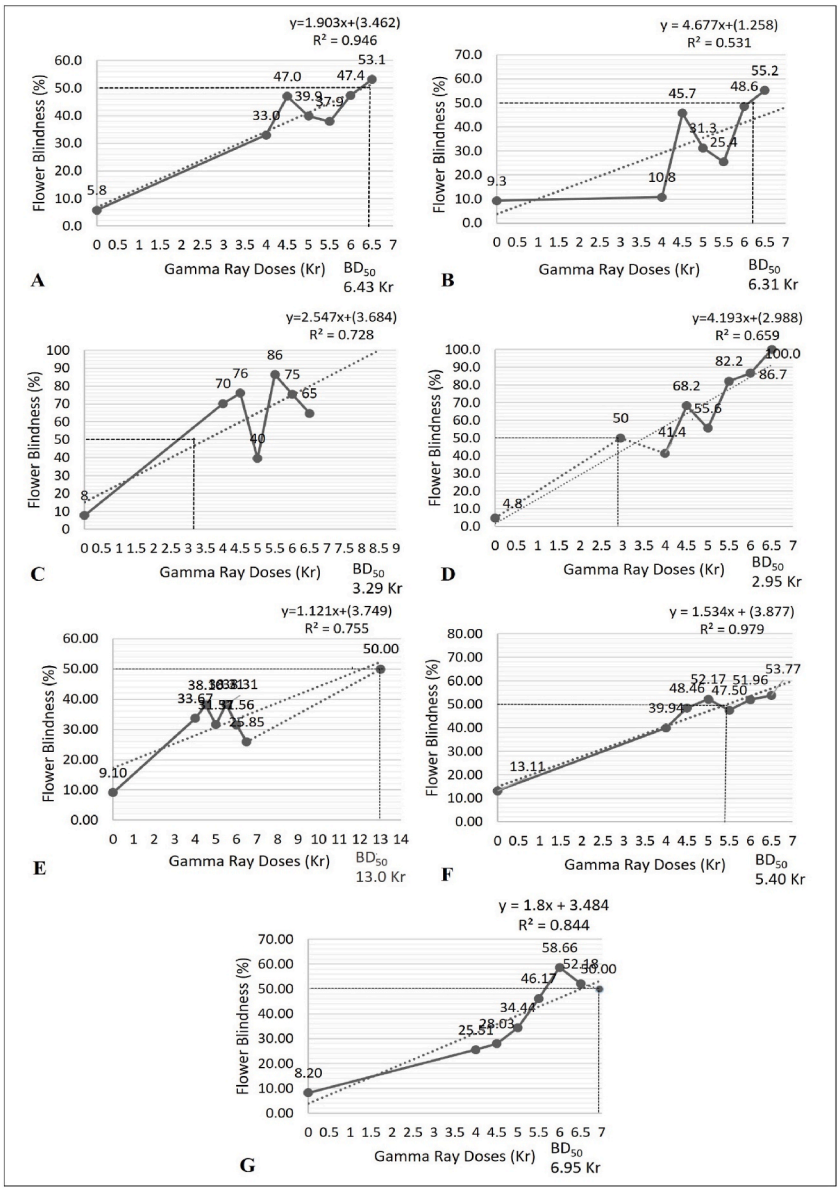

Fig. 2. Patters (A–G). Determination of Blind dose (BD_{50}) of gamma ray doses on different genotypes of gladiolus using probit analysis (A) Nova Lux, (B) Praha, (C) Black Star, (D) Nathan Red, (E) Candyman, (F) Punjab Dawn and (G) Tiger Flame.

4.3. Blind dose (BD_{50})

In the present studies for the genotypes V_1, V_2, V_3, V_4 and V_6 median blind doses (BD_{50s}) were achieved, while for the genotypes V_5 and V_7 to achieve BD_{50} irradiation with higher doses are to be needed in future experiments with higher doses of gamma rays (>6.5 Kr) (Fig. 2A–G). The median blind dose (BD_{50}) was computed on the basis of flower blindness percentage (%) in four consecutive generation as discussed earlier. Determining the median blind dose may be useful in both optimising the radiation dosage for flower mutations and comprehending the impact of ionizing radiation on the flower of the plant under consideration. Flower blindness were also observed by several researcher but not analyzed the median lethal dose before. Patil and Dudhuk [44] observed flower blindness in gladiolus between 6 Kr to 7 Kr dose of gamma rays.

4.4. Percent blind plants (%)

A highly significant interaction between the gamma ray doses and percent blind plants were observed in present study. The flower blind plants was observed highest at G_5 and G_6. Whereas, in untreated plants minor flower blindness was might be due to the lower

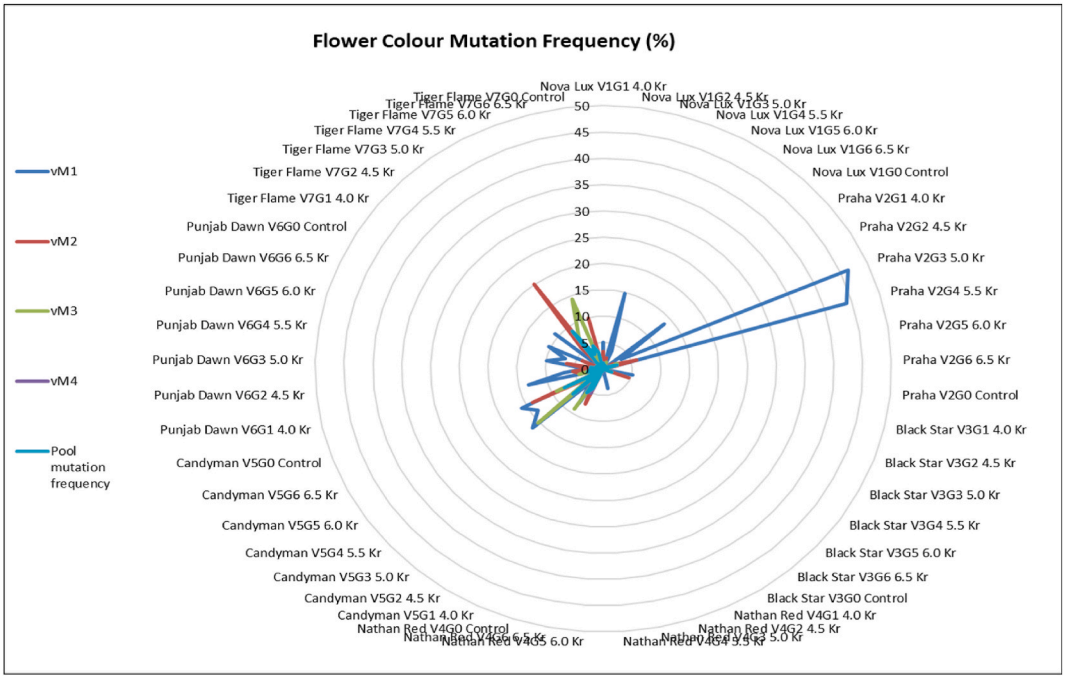

Fig. 3. Flower colour mutation frequency of different treatment combination of seven gladiolus genotypes with seven doses of gamma rays.

temperature during the vegetative growth phase. Gamma doses had showed both reducing and enhancing effect on per cent blind plants in irradiated plant population. However, the increasing impact was more significant than the lowering effect. Similarly, Raghava et al. [42] also observed blind plants in gladiolus after exposing several gladiolus genotypes with 10 Kr of gamma rays. Plants remained blind in gladiolus genotype Eurovision and Nova Lux at 6 Kr and 7 Kr of gamma rays, respectively [44]. Kumari and Kumar [45] found out that the upsurge in blind plant numbers is most likely caused by gamma rays that the emergence of inflorescence

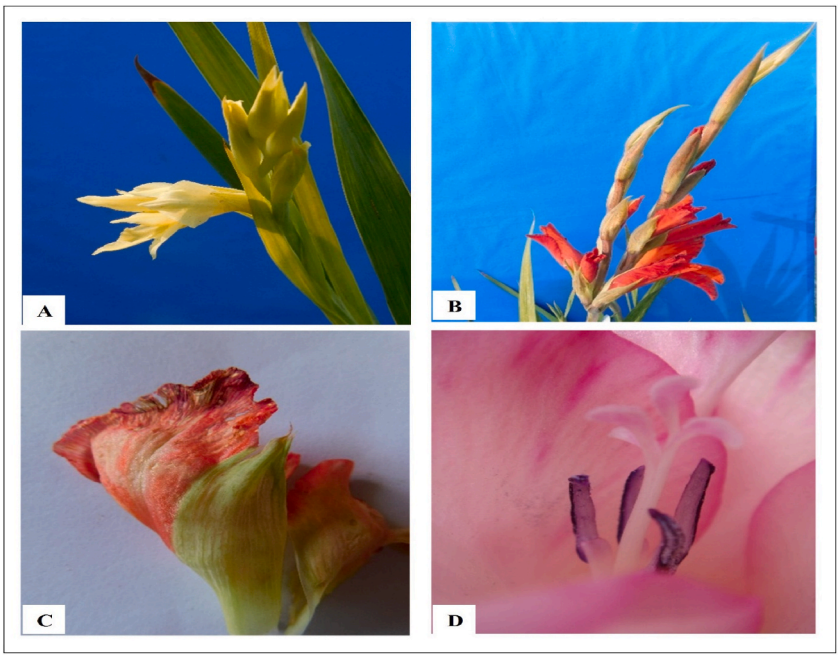

Fig. 4. (Pattern A-D): Vegetative and Floral Abnormality in the plants after exposing with different doses of gamma rays. (A) Shorting of spike in genotype Nova Lux, (B) Bifurcation of spike of genotype Praha, (C) Fusion of sepals and petals in genotype Tiger Flame, (D) Development of one extra stigma and anther in the floret of genotype Candyman.

constantly precedes with the development of particular quantities of leaves in gladiolus.

4.5. Percent abnormal plants (%)

In the current investigation, the unusual pattern in the irradiated and non-irradiated plants showed significant variations for the percent abnormal plants. In case of gamma ray dose, the percent abnormal plants were found highest between the 4.0 Kr (G_1) to 5.5 Kr (G_4) gamma ray doses whereas, in case of genotypes highest abnormal plants were recorded in genotypes V_1 and V_6. The plant and spike abnormality had expressed in the form of shorting of spike, bifurcation of spike, fusion of sepals with petals and development of extra anther and stigma in florets (Fig. 4A–D). The direct and significant correlation were found between the percent abnormal plant and gamma ray doses. After exposing gladiolus cultivars to gamma radiation in the vM_1 generation, Tiwari et al. [36] also noted asymmetrical floret growth, aberrant spikes, and fasciation of buds. According to Donini [46], the spike anomalies in plants might have originated from triggering an excessive number of mutational events, which elevated the probability of both desirable and unwanted genetic variation. Chromosome aberrations, genetic mutations, chromosome number variations, reorganization of distinct histogenic layers and modified biochemical pathways can all cause radiation-induced alterations in flowers, leaves, and other tissues [47].

4.6. Flower colour mutation frequency and spectrum

Very substantial differences in bloom colour and morphology were discovered in the current study. The flower colour mutation frequency (FCMF) was found highest in genotypes Tiger Flame (V_7) at 5.0 Kr (G_3) followed by genotype Candyman (V_5) at 5.0 (G_3) and 5.5 Kr (G_4) dose gamma radiations, while the least variation observed in genotype Nova Lux (V_1) at 5.5 Kr (G_4) (Table 4). Overall Flower colour mutation spectrum was observed between 4.0 Kr (G_1) to 5.5 Kr (G_4) in all genotypes except for genotypes Candyman (V_5) and Tiger Flame (V_7) (4.0 Kr to 6.5 Kr) (Table 4; Fig. 5A–H). The FCMFs were found higher in those genotypes which had the strong flower pigment colours as compare to genotypes had the lighter flower pigments.

The mutations had been observed mostly in the forms of chimeras, variegations, and colour blends in flower spike of gladiolus in

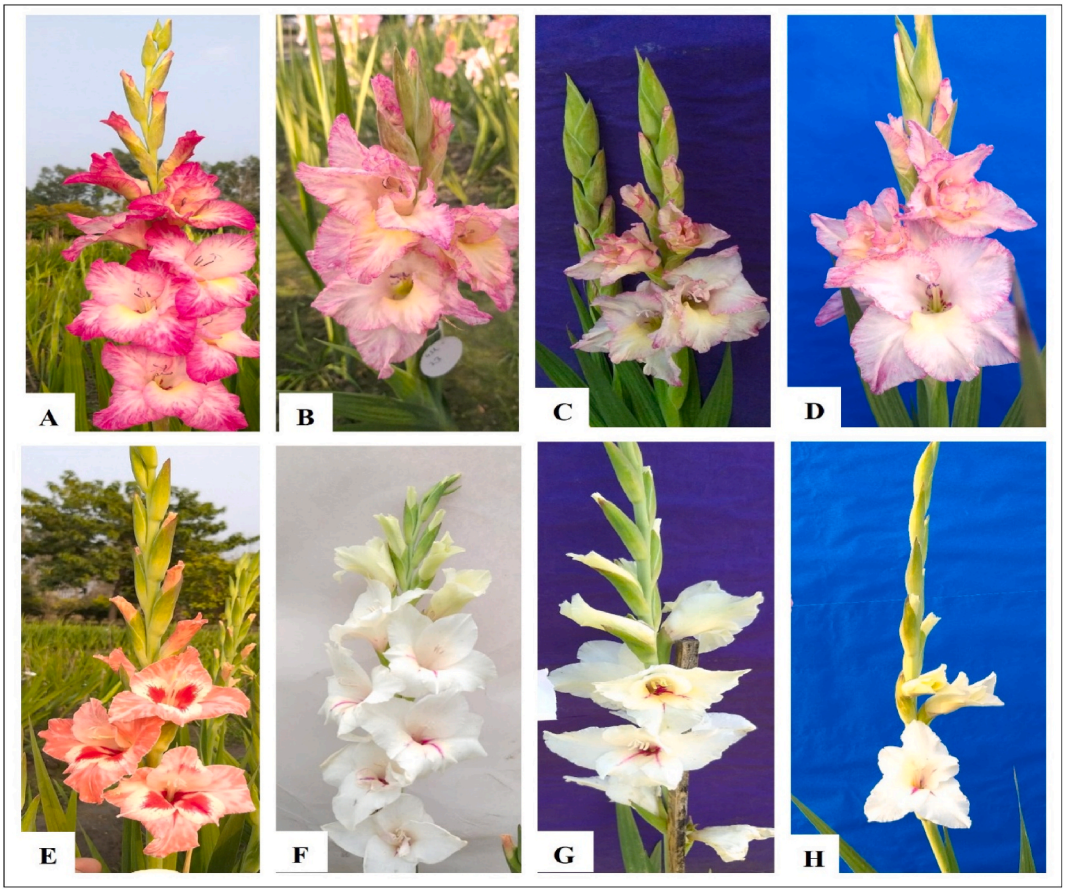

Fig. 5. (Pattern A-H): Strong mutant were isolated from the genotype Candyman (A) and Punjab Dawn (E) and undergone through stability over the generations. Mutant isolated in vM_2 generation at 4.5 Kr and 4.0 Kr dose of gamma rays from genotype Candyman (B) and Punjab Dawn (F), respectively. Reoccurrence of previously isolated mutants in vM_3 (C, G) and in vM_4 generation (D, H) of genotypes Candyman and Punjab Dawn.

subsequent generations in present study. Some of the mutant were also observed for their stability in vM_2, vM_3 and vM_4 generations (Fig. 5A–H). Buiatti et al. [48] reported this kind of findings in gladiolus cv. "Oscar" after treating them with 4. 0 Kr to 6.0 Kr of gamma radiations. They observed that the mutated sector size, form and expressivity were varied from plant to plant and genotype to genotype. Sectorial chimaeras were seen by Ref. [27] in chrysanthemum mutant flowers from the vM_1 generation, which is consistent with the results of this investigation. Kumari and Kumar [45] have discovered similar forms of chimerical patterns following irradiation in various gladiolus cultivars. Periclinal and sectorial chimeric forms of flower colour mutants were observed in gladiolus genotype Sylvia, when irradiated with 2–4 Kr dose of gamma rays" by Ref. [49]. The changes in flower colour was observed in few florets or as well as in whole spike in few genotypes. The gamma irradiation-induced production of colour mutations was lines up with earlier findings in ornamental flower plants such as gladiolus, chrysanthemum, and carnation [50–53].

In this work, we determined the fatal and flower blindness dosages of gamma radiation to several gladiolus cultivars, as well as the frequency and spectrum of mutations. However, further research is required in subsequent studies where we might combine several modern plant improvement approaches and techniques. We might explore for more productive outcomes by targeting phenotypic variations associated to plant genotypes through the combination of mutant breeding and molecular approaches. The recent approaches such as bulked segregant analysis, association mapping, genome re-sequencing, and fine gene mapping identify single base-pair polymorphisms and QTL-linked biomarkers for genome manipulation and germplasm enhancement, and screen for gene mutations [54,55]. Despite its effectiveness, traditional mutagenesis is constrained by the need for extensive screening and the presence of undesired lethal mutations [56]. CRISPR/nCas9 or *dCas9* linked with cytidine deaminase allows targeted point mutations [57], as proven in cotton genome editing using the *GhBE3* system. CRISPR-associated endonucleases, such as *Cpf1*, give precise genome editing with increased specificity [58,59].

5. Conclusion

From the combined study of consecutive generations, it can be concluded that gamma radiation significantly varied with respect to plant survival percentage (%), flower blindness percentage (%), percent abnormal plants (%) and flower colour mutation frequency. The most suitable dose for getting highest plant survivability were found between 4.0 Kr (G_1) and 5.0 Kr (G_3), while for genotype V_5 and V_7 were found most resistant to gamma irradiation exposer over the generations. The radio-sensitivity levels for different genotypes were also achieved by identifying 6.31 Kr and 5.90 Kr as median lethal dose (LD_{50}) for genotype V_3 and V_4, respectively, whereas partial lethal dose (LD_{25}) and median blind dose (BD_{50}) were achieved for all genotypes between 5.0 Kr (G_3) and 6.5 Kr (G_6) and less than 6.5 Kr except for the genotype V_5 and V_7. The percent abnormal plants were recorded highest between the 4.0 Kr (G_1) and 5.5 Kr (G_4) doses of gamma rays and incase of genotypes *i.e.,* V_6 and V_2 had the highest plant abnormality percentage. The highest flower colour mutation frequency was found at G_4 (5.5 Kr) of gamma rays. Radiation doses ranging from 4.0 Kr (G_1) to 5.5 Kr (G_4), as well as genotype V_5 and V_7, were shown to be most successful in generating larger phenotypic variants. Therefore, irradiation with the gamma rays could be utilized as a primary breeding tool for the enhancement of the gladiolus genepool and could be later on aligned with modern biotechnological tools for the development of favorable and desirable variability in gladiolus and other horticultural tools. Similarly, the repeated experiment can be performed in other flower crops to study the influence of gamma irradiation on plant survivability, radiosensitivity, variability and other important agronomic parameters.

Ethical statement

The gamma radiation exposers to plant materials were performed in gamma chamber by authorized person, as per rules and regulations of BARC, Department of Atomic Energy, and Government of India. No animal or human were harmed during the course of investigation. The authors considered all sorts of ethical issues regarding the use of plant materials.

Data availability statement

The original contributions presented in the current study are included in the article, further inquiries can be directed to the corresponding author.

CRediT authorship contribution statement

Anand Singh Rawat: Writing – original draft, Methodology, Investigation, Formal analysis, Conceptualization. **B.D. Bhuj:** Writing – review & editing, Methodology, Investigation, Formal analysis, Conceptualization. **Ranjan Srivastava:** Writing – review & editing, Methodology, Investigation, Conceptualization. **Satish Chand:** Writing – review & editing, Methodology, Conceptualization. **N.K. Singh:** Writing – review & editing, Methodology, Investigation, Data curation, Conceptualization. **Yashpal Singh Bisht:** Writing – review & editing, Formal analysis, Data curation, Conceptualization. **Hemant Dasila:** Writing – review & editing, Formal analysis, Data curation. **Rajendra Bhatt:** Writing – review & editing, Data curation. **Kahkashan Perveen:** Writing – review & editing, Data curation. **Najat A. Bukhari:** Writing – review & editing, Formal analysis, Data curation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to

influence the work reported in this paper.

Acknowledgments

The authors would also like to acknowledge the support provided by Researchers Supporting Project Number RSP2024R358, King Saud University, Riyadh, Saudi Arabia. We also thank to In-charge of Radiations and Isotopic Tracers Laboratory (R.I.T.L.), CBSH, G.B. Pant University of Agriculture and Technology for providing the gamma chamber facility for the irradiation of plant material.

References

[1] S.A. Zafar, M. Aslam, M. Albaqami, A. Ashraf, A. Hassan, J. Iqbal, A. Maqbool, M. Naeem, R. Al-Yahyai, A.T.K. Ali Tan Kee Zuan, Gamma rays induced genetic variability in tomato (*Solanum lycopersicum* L.) germplasm, Saudi J. Biol. Sci. 29 (5) (2022) 3300–3307, https://doi.org/10.1016/j.sjbs.2022.02.008.
[2] C. Konzak, R. Nilan, A. Kleinhofs, Artificial mutagenesis as an aid in overcoming genetic vulnerability of crop plants, in: Genet. Divers. Plants, Springer, 1977, pp. 163–177, https://doi.org/10.1007/978-1-4684-2886-5_15.
[3] B. Ahloowalia, M. Maluszynski, K. Nichterlein, Global impact of mutation-derived varieties, Euphytica 135 (2004) 187–204, https://doi.org/10.1023/B:EUPH.0000014914.85465.4f.
[4] A. Mehandjiev, G. Kosturkova, M. Mihov, Enrichment of *Pisum sativum* gene resources through combined use of physical and chemical mutagens, Isr. J. Plant Sci. 49 (2001) 280–284, https://doi.org/10.1560/UQPC-NNXP-GU0L-P3X4.
[5] FAO/IAEA, Induced plant mutations in the genomics era, in: Q.Y. Shu (Ed.), Joint FAO/IAEA Programme, Division of Nuclear Techniques in Food and Agriculture, International Atomic Energy Agency, Vienna (Austria), Food and Agriculture Organization of the United Nations, Rome, 2009, pp. 262–265 (Italy).
[6] FAO/IAEA, M.M. Lopes, B.P. Spencer, L. Forster, Jankuloski. Manual on Mutation Breeding, third ed., Food and Agriculture Organization of the United Nations, Rome, Italy, 2018.
[7] G.M. Ludovici, S.O. de Souza, A. Chierici, M.G. Cascone, F. d'Errico, A. Malizia, Adaptation to ionizing radiation of higher plants: from environmental radioactivity to chernobyl disaster, J. Environ. Radioact. 222 (2020) 106375, https://doi.org/10.1016/j.jenvrad.2020.106375.
[8] C. Mba, Induced mutations unleash the potentials of plant genetic resources for food and agriculture, Agronomy 3 (2013) 200–231, https://doi.org/10.3390/agronomy3010200.
[9] R. Beyaz, M. Yildiz, The use of gamma irradiation in plant mutation breeding, Plant Eng. 17 (2017) 32–43, https://doi.org/10.5772/intechopen.69974.
[10] S. Gupta, C. Schmitt, K. Mahata, A. Shrivastava, P. Sugathan, A. Jhingan, K. Golda, N. Saneesh, M. Kumar, G. Kaur, Asymmetric fission around lead: the case of Po 198, Phys. Rev. C 100 (2019) 064608, https://doi.org/10.1103/PhysRevC.100.064608.
[11] A.S. Rawat, B.D. Bhuj, R. Srivastava, S. Chand, N.K. Singh, Y.S. Bisht, R. Bhatt, Effect of ionizing radiated gladiolus (*Gladiolus grandifloras*) on vase life attributes of various cultivars, Indian J. Agric. Sci. 94 (3) (2024) 281–285, https://doi.org/10.56093/ijas.v94i3.145057.
[12] S.K. Datta, Gladiolus, in: Role of Mutation Breeding in Floriculture Industry, Springer, Singapore, 2023, https://doi.org/10.1007/978-981-99-5675-3_9.
[13] A. Álvarez-Holguín, C.R. Morales-Nieto, C.H. Avendaño-Arrazate, R. Corrales-Lerma, F. Villarreal-Guerrero, E. Santellano-Estrada, Y. Gómez-Simuta, Mean lethal dose (LD_{50}) and growth reduction (GR_{50}) due to gamma radiation in Wilman lovegrass (*Eragrostis superba*), Rev. Mex. Cienc. Pecu. 10 (2019) 227–238, https://doi.org/10.22319/rmcp.v10i1.4327.
[14] N. Surakshitha, K. Soorianathasundaram, Determination of mutagenic sensitivity of hardwood cuttings of grapes 'Red Globe'and 'Muscat'(*Vitis vinifera* L.) to gamma rays, Sci. Hortic. 226 (2017) 152–156, https://doi.org/10.1016/j.scienta.2017.08.040.
[15] B. Chakravarty, S. Sen, Enhancement of regeneration potential and variability by γ-irradiation in cultured cells of *Scilla indica*, Biol. Plant. (Prague) 44 (2001) 189–193, https://doi.org/10.1023/A:1010282805522.
[16] L. Tshilenge-Lukanda, C. Funny-Biola, A. Tshiyoyi-Mpunga, J. Mudibu, M. Ngoie-Lubwika, R. Mukendi-Tshibingu, A. Kalonji-Mbuyi, Radio-sensitivity of some groundnut (*Arachis hypogaea* L.) genotypes to gamma irradiation: indices for use as improvement, Br. Biotechnol. J. 2 (2012) 169–178, https://doi.org/10.9734/BBJ/2012/1459.
[17] C. Ke, W. Guan, S. Bu, X. Li, Y. Deng, Z. Wei, W. Wu, Y. Zheng, Determination of absorption dose in chemical mutagenesis in plants, PLoS One 14 (2019) e0210596, https://doi.org/10.1371/journal.pone.0210596.
[18] I. Muhammad, M.Y. Rafii, M.H. Nazli, S.I. Ramlee, A.R. Harun, Y. Oladosu, Determination of lethal (LD) and growth reduction (GR) doses on acute and chronic gamma-irradiated Bambara groundnut [*Vigna subterranea* (L.) Verdc.] varieties, J. Radiat. Res. Appl. Sci. 14 (2021) 133–145, https://doi.org/10.1080/16878507.2021.1883320.
[19] E. Domingo, C. Perales, R. Agudo, A. Arias, C. Escarmís, C. Ferrer-Orta, N. Verdaguer, Mutation, quasispecies, and lethal mutagenesis, in: E. Ehrenfeld, E. Domingo, R.P. Roos (Eds.), The Picornaviruses, ASM Press, Washington, DC, 2010, pp. 195–221, https://doi.org/10.1128/9781555816698.ch12.
[20] A.M. Van Harten, Mutation Breeding: Theory and Practical Applications, Cambridge University Press, United Kingdom, 1998, p. 353.
[21] H. Gaul, Critical analysis of the methods for determining the mutation frequency after seed treatment with mutagens, Genet. Agrar. 12 (1960) 297–318.
[22] L. Horn, H. Shimelis, Radio-sensitivity of selected cowpea (*Vigna unguiculata*) genotypes to varying gamma irradiation doses, Sci. Res. Essays 8 (40) (2013) 1991–1997, https://doi.org/10.5897/SRE2013.5682.
[23] K.A. Gomez, A.A. Gomez, Statistical Procedures for Agricultural Research, Wiley-Interscience Publications, New York, NY, USA, 1983, p. 84.
[24] R.G.D. Steel, J.H. Torrie, Principles and Procedures of Statistical Analysis, McGraw Hill Book Co. Inc., New York, NY, USA, 1980, pp. 232–251.
[25] J.R. Busvine, A Critical Review of the Techniques for Testing Insecticides, second ed., Commonwealth Agricultural Bureaux, Slough, UK, 1971, p. 345.
[26] C. Ritz, F. Baty, J.C. Streibig, D. Gerhard, Dose-response analysis using R, PLoS One 10 (2015) e0146021, https://doi.org/10.1371/journal.pone.0146021.
[27] R.L. Misra, Radiation induced variability in gladioli, Indian J. Genet. 58 (2) (1998) 237–239.
[28] M.S. Swaminathan, V.L. Chopra, S. Bhaskaran, Chromosome aberrations and the frequency and spectrum of mutations induced by ethylmethane sulphonate in barley and wheat, Indian J. Genet. 22 (1962) 192–207.
[29] R. Singh, J.S. Jaya Singh, Genotypic variability relationship with EMS in a Trigonella species complex, Indian J. Hortic. 46 (1989) 111–114.
[30] N. Tuteja, M.B. Singh, M.K. Misra, P.L. Bhalla, R. Tuteja, Molecular mechanisms of DNA damage and repair: progress in plants, Crit. Rev. Biochem. Mol. Biol. 36 (4) (2001) 337–397, https://doi.org/10.1080/20014091074219.
[31] D.I. Roslim, I. Fiatin, Lethal dose 50 (LD 50) of mungbean (*Vigna radiata* L. Wilczek) cultivar Kampar, SABRAO J. Breed. Genet. 47 (2015) 510–516.
[32] D. Arpitha, S. Nataraj, V. Srinivasa, M. Ganapathi, A. Ramesh, Assessment of survivability and genetic variability in mutated population of hybrid cut Chrysanthemum cv. Lexy red, ecology, Environ. Conserv. 29 (2023) 206–209, https://doi.org/10.53550/EEC.2023.v29i02s.037.
[33] M. Singh, M. Bala, Induction of mutation in chrysanthemum (*Dendranthema grandiflorum* Tzvelev.) cultivar Bindiya through gamma irradiation, Indian J. Hortic. 72 (2015) 376–381, https://doi.org/10.5958/0974-0112.2015.00073.0.
[34] K. Ussuf, P. Nair, Effect of gamma irradiation on the indole acetic acid synthesizing system and its significance in sprout inhibition of potatoes, Radiat. Bot. 14 (1974) 251–256, https://doi.org/10.1016/S0033-7560(74)80015-3.
[35] A. Tanaka, N. Shikazono, Y. Hase, Studies on biological effects of ion beams on lethality, molecular nature of mutation, mutation rate, and spectrum of mutation phenotype for mutation breeding in higher plants, J. Radiat. Res. 51 (3) (2010) 223–233, https://doi.org/10.1269/jrr.09143.
[36] M. Justin, K. Kabwe, K.-M. Adrien, V.K. Roger, Effect of gamma irradiation on morpho-agronomic characteristics of soybeans (*Glycine max L.*), Am. J. Plant Sci. 2012 (2012), https://doi.org/10.4236/ajps.2012.33039.
[37] C. Ojiewo, S. Agong, K. Murakami, S. Tanaka, Y. Hase, M. Masuda, Male-sterility induced by gamma-ray irradiation of African nightshade (*Solanum nigrum* L. ssp. villosum) seed, J. Hortic. Sci. Biotechnol. 80 (2005) 699–704, https://doi.org/10.1080/14620316.2005.11512001.

[38] D. Kpm, R. Vrk, Cytogenetic effects of gamma rays and ethyl methane sulphonate in chilli pepper (*Capsicum annuum* L.), Cytologia 65 (2000) 129–133, https://doi.org/10.1508/cytologia.65.129.
[39] Z. Tadele, Mutagenesis and Tilling to dissect gene function in plants, Curr. Genom. 17 (2016) 499–508, https://doi.org/10.2174/1389202917666160520104158.
[40] V. Manova, D. Gruszka, DNA damage and repair in plants–from models to crops, Front. Plant Sci. 6 (2015) 1–26, https://doi.org/10.3389/fpls.2015.00885.
[41] G. Mallick, R.S. Pramanik, M.K. Pandit, A.K. Gupta, S. Roy, S. Jambhulkar, S. Bhattacharyya, Radiosensitivity of seedling traits to varying gamma doses, optimum dose determination and variation in determined doses due to different time of sowings after irradiation and methods of irradiation in faba bean genotypes, Int. J. Radiat. Biol. 99 (3) (2022) 534–550, https://doi.org/10.1080/09553002.2022.2107723.
[42] S.P.S. Raghava, S.S. Negi, T.V.R.S. Sharma, K.A. Balakrishnan, Gamma ray induced mutants in gladiolus, J. Nucl. Agric. Biol. 17 (1) (1988) 5–10.
[43] P. Rajasekar, M. Kannan, M. Kumar, Effect of physical and chemical mutagens in chrysanthemum (*Dendranthema grandiflora* Tzvelev) cv. Jaya for determination of mutagenic sensitivity, Intenational J, Pure Appl. Biosci. 7 (2) (2019) 235–241, https://doi.org/10.18782/2320-7051.7183.
[44] S. Patil, B.K. Dhaduk, Effect of gamma radiation on vegetative and floral characters of commercial varieties of gladiolus (*Gladiolus hybrida* L.), J. Orna. Horti. 12 (4) (2009) 232–238.
[45] K. Kumari, S. Kumar, Effect of gamma irradiation on vegetative and propagule characters in gladiolus and induction of homeotic mutants, Int. J. Agric. Environ. Biotechnol. 8 (2015) 413–422, https://doi.org/10.5958/2230-732X.2015.00049.2.
[46] B. Donini, Induction and isolation of somatic mutations in vegetatively propagated plants, in: Improvement of Vegetative Propagated Plants through Induced Mutations. Proc of a Res Co-ordination Meeting Held at Tokai, 30 September to 4 October 1974, Organized by the Joint FAO/IAEA Division of Atomic Energy in Food and Agriculture, 1975, pp. 35–51. IAEA-173 Technical document Issued by the IAEA Vienna.
[47] M. Bala, K.P. Singh, Gamma irradiation of in-vitro proliferated cultures of rose (*Rosa hybrida*) for induction of novel mutants, Indian J. Agric. Sci. (2016) 137–142.
[48] M. Buiatti, R. Tesi, M. Molino, A developmental study of induced somatic mutations in Gladiolus, Radiat. Bot. 9 (1969) 39–48, https://doi.org/10.1016/S0033-7560(69)80020-7.
[49] U.S. Kaicker, H.P. Singh, Somatic mutations in gladiolus, in: Abstract of Contributed Paper, in XV International Congress of Genetics, New Delhi, Dec.1983, pp. 12–21.
[50] K.P. Singh, B. Singh, S.P.S. Raghava, C.S. Kaua, Induced flower colour mutations in carnation through in vitro application of chemical mutagen, Indian J. Genet. Plant Breed. 60 (4) (2000) 535–539.
[51] T. Nonomura, Y. Ikegami, Y. Morikawa, Y. Matsuda, H. Toyoda, Induction of morphologically changed petals from mutagen-treated apical buds of Rose and plant regeneration from varied petal-derived calli, Plant Biotechnol. 18 (2001) 233–236, https://doi.org/10.5511/plantbiotechnology.18.233.
[52] T.B. Dao, P.D. Nguyen, Q.M. Do, T.H. Vu, T.H. Le, T.L.K. Nguyen, H.D. Nguyen, X.L. Nguyen, *In vitro* mutagenesis of chrysanthemum for breeding, Plant Mutation Reports 1 (2) (2006) 26–27.
[53] H. Yamaguchi, A. Shimizu, K. Degi, T. Morishita, Effects of dose and dose rate of gamma ray irradiation on mutation induction and nuclear DNA content in chrysanthemum, Breed Sci. 58 (2008) 331–335, https://doi.org/10.1270/jsbbs.58.331.
[54] T.L. Wang, C. Uauy, E. Robson, B. Till, TILLING in extremis, Plant Biotechnol. J. 10 (2012) 761–772.
[55] C. Zou, P. Wang, Y. Xu, Bulked sample analysis in genetics, genomics and crop improvement, Plant Biotechnol. J. 14 (2016) 1941–1955.
[56] M.E. Bolger, B. Weisshaar, U. Scholz, N. Stein, B. Usadel, K.F. X Mayer, Plant genome sequencing-Applications for crop improvement, Curr. Opin. Biotechnol. 26 (2014) 31–37.
[57] C.M. McCallum, L. Comai, E.A. Greene, S. Heniko, Targeted screening for induced mutations, Nat. Biotechnol. 18 (2000) 455.
[58] S. Li, X. Zhang, W. Wang, X. Guo, Z. Wu, W. Du, Y. Zhao, L. Xia, Expanding the scope of CRISPR/Cpf1-mediated genome editing in rice, Mol. Plant 11 (2018) 995–998.
[59] L. Qin, J. Li, Q. Wang, Z. Xu, L. Sun, M. Alariqi, H. Manghwar, G. Wang, B. Li, X. Ding, High E cient and precise base editing of CG to TA in the allotetraploid cotton (*Gossypium hirsutum*) genome using a modified CRISPR/Cas9 system, Plant Biotechnol. J. 18 (2020) 45–56.