Induction of genetic variability with gamma radiation and detection of DNA polymorphisms among radiomutants using sequence-related amplified polymorphism markers in *Gaillardia pulchella* Foug. plants

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Received: 30 December 2022
Revised: 1 February 2023
Accepted: 1 February 2023
Published: 27 June 2023

Egyptian Pharmaceutical Journal 2023, 22:272–284

Background
Developing novel ornamental varieties with improved floral characterization is the main aim of floriculture. Biotechnological techniques linked to classical breeding methods have been applied for modifying flower color.
Objective
This investigation was carried out in the nursery of the Ornamental Horticulture Department, Faculty of Agriculture, Cairo University, Egypt, during two successive generations, 2019/2020 and 2020/2021, to assess the effects of gamma irradiation (γ) on vegetative growth, flowering parameters, abnormalities, and induced changes at the DNA level between two mutative generations (MG1 and MG2) of *Gaillardia pulchella* Foug. plants.
Materials and methods
Seeds of *G. pulchella* (local red) were irradiated at Atomic Energy Commission-united irradiation-Gamma, The *Egyptian Atomic Energy* Authority (*EAEA*), Nasr City, Cairo, Egypt, by six doses of γ-irradiation (10, 20, 30, 40, 50, and 60 Gy), using Gamma-1 type cobalt^{60}, at a dose rate of 1.107 KGy/h.
Results and conclusion
The results revealed that low gamma doses (10 and 20 Gy) had significant effects on vegetative growth, that is, plant height and the number of branches, as compared with the control, giving the tallest plants with the highest number of branches. The high doses (50 and 60 Gy) delayed flowering compared with untreated plants and other gamma doses. In contrast, low doses induced early flowering and increased the number of flowers. All doses of gamma rays induced mutants in leaf morphology, inflorescence color, shape, and deformation; the largest number of these mutants was obtained from a high dose of 60 Gy. On the contrary, sequence-related amplified polymorphism analysis produced 32 loci, of which 12 (37.50%) were polymorphic. Jaccard's coefficients of dissimilarity ranged from 0.69 to 0.96. In a dendrogram constructed depending on genetic identity coefficients, the mutants were classified into three major groups: the first group (I) was composed of 10-, 20-, 30-, and 40-Gy mutants. The second group (II) included 50- and 60-Gy mutants. The third group (III) contained only the control. Therefore, it was concluded that treatment of *G. pulchella* seeds with gamma rays led to the induction of a sufficient number of mutations. In addition, the sequence-related amplified polymorphism marker is considered to be an important tool in the identification of mutants. Consequently, these mutants can be used in breeding programs to improve *G. pulchella* plants.

Keywords:
abnormalities, cluster analysis, flowering mutants, gamma rays, sequence-related amplified polymorphism-PCR

Egypt Pharmaceut J 22:272–284

1687-4315

Introduction

Gaillardia pulchella is a kind of Gaillardia. It is a flowering plant in the *Asteraceae* family. A genus of *Gaillardia* is an annual, biennial, perennial herbs, and subshrub with about 25–30 species native to the Americas. The name 'blanket flower' refers to the colors of the wild species' blossoms, which completely cover the land [1]. All *Gaillardia* species have flowers with a central disc of many small flowers encompassed by 15 or more sterile ray flowers. The ray flowers are normally long and flat, like petals with

three-toothed tips. The flower head is single or double. The ray flowers often have bands of color. The flower heads are 2–4 inches in diameter and bloom from April to September. The leaves are gray–green, alternating, large, hairy, and soft. The edges might be smooth, toothed, or lobed, and they can all appear on the same plant. The compact varieties look great massed in front of borders. They can be grown in pots, alone, or incorporation with drought-tolerant plants; the taller varieties make nice cut flowers [2]. The *Gaillardia* plant is also considered an important medicinal plant owing to the many components it contains with pharmaceutical uses; it was found that the inflorescence extract contains many chemical compounds, such as flavonoids, polysaccharides, amino acids, pectin, and tannins, which have important pharmaceutical uses. The amino acids identified are aspartic and glutamic acids, α-alanine, α-proline, methionine, and valine [3].

Gamma (γ) rays are one of the most common physical mutagens that cause mutations in plants. It has a negative effect on plant characteristics, which varies depending on the irradiation dose and the plant species or cultivars [4]. Mutagenesis by gamma irradiation plays a main role in the generation of novel mutagens with enhanced characterization, which can generate higher quantities of commercially important metabolites. Gamma irradiation resulted in many beneficial mutations in agricultural crops and was successfully used in developing crops with improved traits [5]. In general, gamma rays may cause some mutations in cell genes via the cell's DNA repair systems [6]. It has a biological effect because it interacts with atoms or molecules in the cell. These radicals can change or damage essential compounds in plant cells, and they have been shown to have varied effects on plant morphology and biochemistry depending on the level of irradiation [7]. The mutagen treatment damages nuclear DNA, and during the DNA repair process, novel mutations appear at random and are heritable. Alterations in cytoplasmic organelles can result in chromosomal or genomic mutations, allowing plant breeders to select desirable mutants for traits including flower color, early flowering types, and flower morphology [8]. Radiation-induced mutation has effectively produced a large number of new varieties in many seeds and ornamental plants and is widely regarded as a highly effective method for ornamental plant breeding [9]. The gamma irradiation mutation breeding method is an effective breeding technology that can alter the DNA sequence of chromosomes, cause DNA sequence deletions, etc., but there is no insertion of novel genes.

Molecular markers such as sequence-related amplified polymorphism (SRAP), which have just recently been discovered, can selectively amplify DNA coding regions by using primers to target an open reading frame [10]. SRAP markers have been widely used in several species since they were indicated in 2001 [11], and they have been reported to be highly stable, efficient, and suitable for direct use in different crops that lack genomic sequencing information. The work aimed to investigate the effects of different doses of gamma irradiation on inducing mutations to improve the growth and flower color of *G. pulchella* plants. Furthermore, we used SRAP profiles to detect DNA polymorphism in mutated and unmutated plants.

Materials and methods

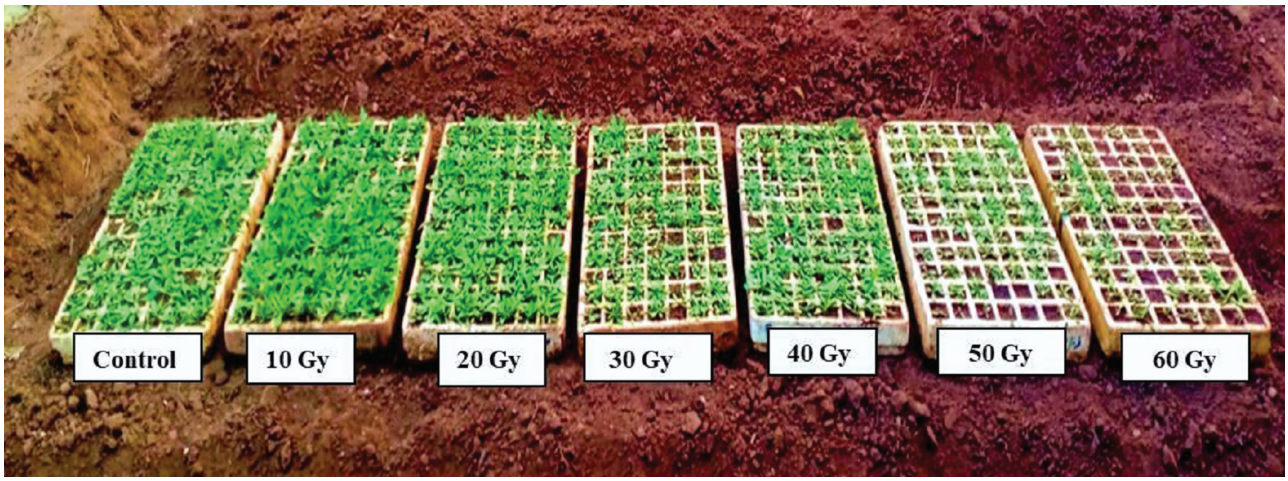
Plant materials

The seeds of *G. pulchella* (the local variety) were obtained from a bred strain in the Ornamental Horticulture Department, Faculty of Agriculture, Cairo University, Egypt. A field experiment was conducted in this location during the two successive seasons of 2019/2020 and 2020/2021 for two generations (MG1 and MG2).

Treatment of seeds

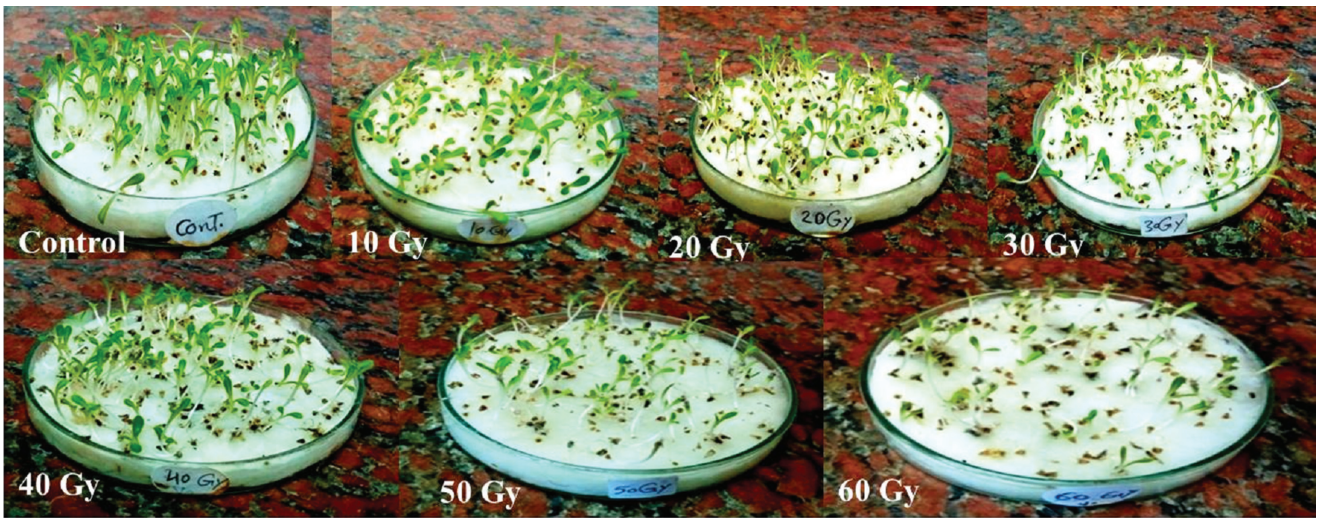
The seeds were presoaked in distilled water (wet seeds) for 1 h before being irradiated with gamma radiation at the Atomic Energy Commission-united irradiation-Gamma, The *Egyptian Atomic Energy Authority* (*EAEA*), Nasr City, Egypt, at doses of 10, 20, 30, 40, 50, and 60 Gy; the control seeds were kept without irradiation. Gray gamma-irradiation doses using gamma-1 type, cobalt 60 (Co^{60}), using an Indian gamma cell (Ge 4000 A), at a dose rate of 1.107 KGy/h, were used. Each treatment had 500 seeds. Immediately after irradiation treatments, 300 seeds were chosen to measure the percentage of germination seeds in the laboratory (Fig. 2). The remaining 200 seeds were sown in plastic trays filled with a mixture of peat moss, loam, and sand (1 : 1 : 1 by volume) on October 5, 2019, and October 5, 2020 (for MG1 and MG2 generations, respectively) (Fig. 1) to produce the seedlings. The seed germination began after 10 days of sowing. After 45 days of sowing, uniform *Gaillardia* seedlings (8–10 cm height), the seedlings of each treatment were transplanted into the open field (clay loam soil), in three rows at 60 cm apart and 50 cm between the hills within each

Figure 1



The seedlings of *Gaillardia pulchella* in plastic trays as affected by different doses of gamma rays.

Figure 2



The seed germination (%) of *Gaillardia pulchella* in Petri dishes as affected by different doses of gamma rays.

row (two plants/hill), as every plot (3.5×1.8 m) contained 21 hills/plot.

Soil analysis

Soil analysis indicated that the particle size distribution (%) was as follows: sand, 26.2; silt, 26.3; and clay, 38.1 (texture : clay loam), with pH of 7.5 and EC ds/m^{-1} of 0.94. Soluble cations (mEq/l) were Na+: 0.70, K+: 0.4, Ca++: 1.3, and Mg++: 0.9, and soluble anions (mEq/l) were Cl: 1.5, HCO_3: 0.74, CO_3: 0, and SO_4: 1.06.

The first and second mutative generations (MG1 and MG2)

The mass selection of plants of the MG1 generation was run from June to July 2019, which focused on some morphological and flowering traits. All plants in the field in each treatment were evaluated, selected, and selfed to obtain the seeds for the second mutative generation (MG2). Frequent observations were taken during the vegetative and flowering stages to detect any variations. The seeds of all the MG1 viable plants that survived were harvested separately for each treatment, when they reached maturity (June to July) and used to grow MG2 generation (seedling) plants. In both generations, all the recommended cultural practices, namely, irrigation and fertilizer, were carried out during the plant's growth and flowering periods. The fertilizers were supplied for each plot as recommended, using Kristalon mineral fertilizer (N : P : K) (19 : 19 : 19). The plants were fertilized monthly

after a month of transplanting (1 g/hill). Irrigation was done with tap water according to the amount of water needed, and weeding was done as the soil required.

Data recorded

The following data were collected from *G. pulchella* plants that were grown until 50% of the flowers were opened, that is, about a month after the flowers start to appear for each treatment: (a) seed germination (%); (b) vegetative parameters [plant height (cm) and number of main branches/plan]; (c) flowering parameters (number of days from planting to flowering), number of flowers (inflorescences)/plant, and flower diameter (cm); and (d) (plant abnormalities) leaves, and inflorescence abnormalities of *G. pulchella* as affected by different doses of gamma.

Extraction of genomic DNA

Overall, 0.5 g of fresh young *G. pulchella* L. leaves was collected from the MG2 generation individuals mutated with gamma irradiation and unmutated ones and were extracted with the DNA purification kit (Bio Basic Inc., Markham, Canada) following the manufacturer's instructions.

Sequence-related amplified polymorphism markers of plant mutant with gamma radiation

Six SRAP primers were designed following Li and Quiros [10] and were used to search for polymorphisms among *G. pulchella* plants mutated by γ-rays and their corresponding controls (Table 1). The total reaction mixture was 25 µl, containing 10× PCR buffer, 2 mM $MgCl_2$, 0.2 mM dNTPs mixed, 10 pmol primers, 1.25 U *Taq* polymerase, and about 150 ng genomic DNA. The amplification regimen followed the recommendations of Li and Quiros, [10] as follows: an initial denaturing step was performed at 94°C for 5 min, followed by five cycles at 94°C for 1 min, 35°C for 1 min, and 72°C for 1 min, subsequently followed by 35 cycles at 94°C for 1 min, 50°C for 1 min, and 72°C for 1 min, with a final extension step at 72°C for 7 min. Amplification products were separated on 1.5% agarose gel containing 1× TBE buffer (89 mM Tris-HCl, 89 mM boric acid, 2.5 mM EDTA, pH 8.3), and 0.5 µg/ml ethidium bromide at 90 V.

Data analysis

A matrix for SRAP was generated by scoring reproducible bands as 1 for their presence and as 0 for their absence across the genotype. Genetic similarity coefficients were computed according to Nei and Li [12]. A dendrogram based on Jaccard similarity coefficients was constructed using the unweighted pair group method of arithmetic averages [13], employing sequential, agglomerative hierarchic and nonoverlapping clustering. All the computations were carried out using the software NTSYS-PC (Numerical Taxonomy and Multivariate Analysis System), version 2.02 [14]. Correlation coefficients were calculated using similarity coefficients obtained from SRAP analysis.

Statistical analysis

A randomized complete block design was used. Three replicates were used for each treatment, and each replicate had nine plants. The results of the trial

Table 1 Sequence of primers used in this the study

Primers names	Sequence	
	Forward	Reverse
SRAP-1	TGAGTCCAAACCGGTAG	GACTGCGTACGAATTGTC
SRAP-2	TGAGTCCAAACCGGTAG	GACTGCGTACGAATTCGA
SRAP-3	TGAGTCCAAACCGGTCC	GACTGCGTACGAATTCAG
SRAP-4	TGAGTCCAAACCGGTCA	GACTGCGTACGAATTCTG
SRAP-5	TGAGTCCAAACCGGTCA	GACTGCGTACGAATTAAT
SRAP-6	TGAGTCCAAACCGGTGC	GACTGCGTACGAATTGTC

The selective nucleotide sequences for each primer are underlined.

Table 2 Significance of means squares due to source of variation for studied parameters in two generations (analysis of variance table)

		Mean squares											
		Seed germination (%)		Plant height (cm)		Number of main branches/plant		Days to flowering		Number of flowers/ plant		Flower diameter (cm)	
S.O.V.	DF	MG1	MG2	MG1	MG2	MG1	MG2	MG1	MG2	MG1	MG2	MG1	MG2
Rep.	2	13.47ns	1.71ns	0.43ns	15.12ns	0.79	1.79ns	29.71**	16.57**	35.65	23.19ns	0.01ns	0.04ns
Treat.	6	524.98**	564.54**	745.08**	560.35**	19.02**	18.98**	623.71**	549.53**	712.06**	914.94**	3.22**	5.02**
Error	12	11.36	6.99	6.41	6.99	1.60	1.23	1.04	0.27	14.33	13.95	0.01	0.02

ns: not significant. ** highly significant.

were statistically analyzed using the Snedecor and Cochran's [15] approach, in which the means were separated using Duncan's [16] and compared using the least significant difference test at 0.05 probability level, with significance determined at *P* value less than 0.05. Statistical one-way analysis of variance was conducted using COSTAT software (Table 2).

Results and discussion

Seed germination (%)

The data recorded in Tables 3 and 4 and Fig. 2 showed that γ-rays had a substantial effect on seed germination, and the highest percentage was recorded in the untreated (control) plants in both MG1 and MG2, followed by the plants treated with γ-rays at the low doses of 10 and 20 Gy (80.33 and 78.33% and 85.00 and 84.00%) in MG1 and MG2, respectively. On the contrary, the lowest percentage of germination rate was scored at the dose of 60 Gy in MG1 (46.66%) and MG2 (52.00%). In this work, it was observed that the doses of γ-rays from 30 to 60 Gy caused significant decreases in the germination rate (%) in both generations, which was attributed to a disturbance on both physical and physiological levels. Our results agreed with those reported by Melki and Marouani [17], who mentioned that the decrease in the percentage of germination at high mutagen dosages could be due to cellular disorders including damage to chromosomes. In contrast, small dosages of gamma rays have been shown to induce the germination of *Atropa belladonna* seeds for a variety of reasons, for example, the acceleration of RNA or protein synthesis, which takes place during the early stages of germination [18]. The decrease in seed germination caused by high dosages may be attributed to seed tissue injury, a decrease in seed moisture content, degradation of meristematic cells, chromosome damage, and mitotic delay. The findings are in agreement with those reported by El-Khateeb *et al.* [19] on *Helichrysum bracteatum* plants. They subjected seeds to eight different doses of radiation (5–40 Gy), and the study revealed that all doses of gamma reduced the germination of seeds in M1 and M2 generations, compared with the control. Chedeo and Wamaedeesa [20] reported on garden balsam (*Impatiens balsamina*). Gamma radiation doses of 0, 50, 100, 200, and 300 Gy were applied to the seeds. All gamma radiation doses reduced seed germination.

Vegetative parameters

Plant height

The obtained data in Tables 3 and 4 revealed that low doses of gamma rays led to a significant increase in

Table 3 Effect of gamma radiation treatments on seed germination (%), plant height (cm), number of main branches/plant, days from planting to flowering, number of flowers/plant and flower diameter (cm) of *Gaillardia pulchella* plant, during the MG1 generation (2019/2020)

Characters treatments	Seed germination (%)	Plant height (cm)	Number of main branches/plant	Days to flowering	Number of flowers/ plant	Flower diameter (cm)
0 Gy (control)	84.00 a	110.07 c	12.18 b	171.00 c	95.52cd	5.48 c
10 Gy	80.33 a	130.03 a	16.95 a	153.00 f	120.66 a	7.20 a
20 Gy	78.33 a	125.96 a	14.74 a	150.00 g	118.92 a	7.16 a
30 Gy	63.66 b	120.60 b	12.33 b	167.00 d	99.73 c	5.49 c
40 Gy	66.00 b	117.71 b	14.89 a	162.00 e	107.47 b	6.37 b
50 Gy	60.33 b	92.05 d	11.12 bc	181.00 b	90.24 d	5.38 c
60 Gy	46.66 c	90.48 d	9.64 c	190.00 a	78.00 e	4.37 d

Values followed by the same letter are not significantly different according to Duncan's multiple range test ($P<0.05$).

Table 4 Effect of gamma radiation treatments on seed germination (%), plant height (cm), number of main branches/plant, days from planting to flowering, number of flowers/plant and flower diameter (cm) of *Gaillardia pulchella* plant, during the MG2 generation (2020/2021)

Characters treatments	Seed germination (%)	Plant height (cm)	Number of main branches/plant	Days to flowering	Number of flowers/ plant	Flower diameter (cm)
0 Gy (control)	89.33 a	116.90 b	14.20 b	160.00 c	105.39 c	5.66 c
10 Gy	85.00 ab	126.09 a	18.82 a	150.20 e	126.85 a	7.77 a
20 Gy	84.00 bc	129.83 a	17.36 a	149.30 e	122.69 ab	7.68 a
30 Gy	79.00 d	115.40 b	14.80 b	160.20 c	104.60 c	5.80 c
40 Gy	80.00 cd	112.87 b	15.15 b	157.20 d	116.19 b	6.73 b
50 Gy	61.66 e	95.10 c	13.68 b	177.20 b	81.78 d	5.17 d
60 Gy	52.00 f	94.98 c	11.08 c	185.26 a	85.41 d	4.30 e

Values followed by the same letter are not significantly different according to Duncan's multiple range test ($P<0.05$).

plant height, whereas higher doses induced a significant decrease in the plant height of both generations, MG1 and MG2. In MG1, the tallest plants were recorded by treating seeds with gamma radiation at 10 Gy (130.03 cm), with an increment of 18.13%, followed by plants treated with 20, 30, and 40 Gy (125.96, 120.60, and 117.71 cm), with an increments of 14.43, 9.56, and 6.94%, respectively, in MG1, as compared with control plants (110.07 cm). However, in the MG2, 20 Gy, followed by plants treated with 10 Gy, induced a significant increase in the height of plants (129.83 and 126.09 cm), with an increment of 11.06 and 7.86%, respectively as compared with controls (116.90 cm). In contrast, the shortest plants in height were recorded in plants treated by the highest levels of gamma radiation at 50 and 60 Gy in both generations (92.05 and 90.48 cm, respectively, in MG1 and 95.10 and 94.98 cm, respectively, in MG2), with a decrement of 16.37 and 17.79%, respectively, in MG1 and 18.64 and 18.75%, respectively, in MG2. The results found are consistent with those acquired by Minisi *et al.* [21], who investigated the influence of gamma radiation on *Moluccella laevis* L. seeds, at doses of 0, 2.5, 5, 7.5, 10, 12.5, 15, 17.5, and 20 Kr. According to the findings, low doses increased plant height, whereas higher doses decreased it. Moreover, *Philodendron scandens*, *Lathyrus odoratus*, *Tagetes erecta*, *H. bracteatum*, *Jasminum grandiflorum*, and *Curcuma heyneana* rhizomes treated with low doses of gamma rays improved plant height and the number of branches per plant [19,22].

The number of main branches/plant

It could be inferred from the results in Tables 3 and 4 that low levels of gamma radiation application significantly increased the number of main branches/plant in both seasons, as compared with the control. The highest values of the number of main branches were recorded in the plants mutated with 10 Gy (16.95), with an increment 39.16% in the first season, followed by doses of 40 Gy (14.89) and 20 Gy (14.74), with increments of 22.24 and 21.01%, respectively, compared with untreated plants (12.18). In the second season, it was shown that two dosages of 10 and 20 Gy led to a significant increase in the number of branches (18.82 and 17.36), with an increment of 32.53 and 22.25%, respectively, compared with the untreated ones (14.20). On the contrary, a dose of 60 Gy caused a significant decrease in the number of main branches in both generations. It gave lower values (9.64 and 11.08), with a decrement of 20.85 and 21.97% in MG1 and MG2, respectively, compared with the control. In this regard, Pitirmovae [23] mentioned that low doses of gamma rays increased vegetative growth, owing to cell division or cell elongation, such as alterations in metabolic operations that affect the synthesis of nucleic acid or phytohormone. This decrease in the growth of plants may be attributed to high dosages of gamma rays, which induce physiological injury to plant tissues or cells, resulting in a switch from normal to stunted growth [24]. Different authors illustrated the effects of higher doses of gamma rays on vegetative growth attributed to several factors, such as an increase in growth inhibitors, alterations in ascorbic acid concentration, inhibition of auxin synthesis, biochemical and physiological abnormalities of metabolites, and a reduction in the absorption operation. All this led to the stunted growth of plants [25].

Flowering parameters

The number of days from planting to flowering

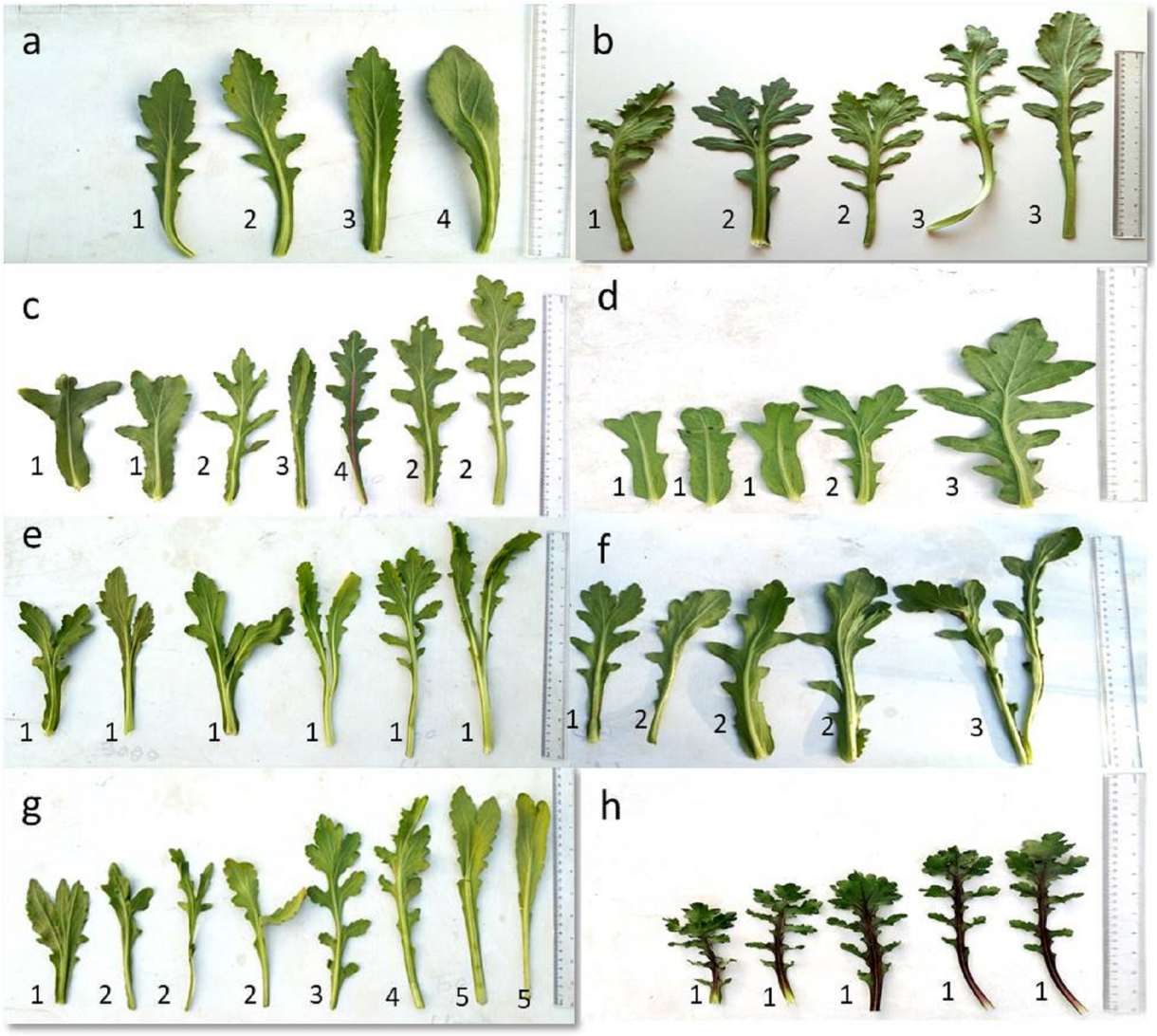
The data recorded in Tables 3 and 4 indicated that the average number of days from the planting of *G. pulchella* L. seeds until the beginning of flowering (days from planting to flowering) was affected by the γ-rays in both seasons. The results of the study found that four different doses of γ-irradiation (10, 20, 30, and 40 Gy) caused significant effects on flower date (earlier flowering) by 153.00, 150.00, 167.00, and 162.00 days, as compared with the control plants (171.00 days). For MG2, three doses of 10, 20, and 40 Gy induced earlier flowering by 150.20, 149.30, and 157.20 days, respectively compared with the control (160.00 days). In contrast, two doses of 50 and 60 Gy delayed flowering by 181.00 and 190.00 days, respectively, in MG1 and 177.20 and 185.26 days, respectively, in MG2, compared with the untreated control. In this investigation, the reaction to mutagen doses or environmental variations can explain the differences between early flowering and late flowering; low and intermediate levels of these mutagens are known to enhance cell development, accelerate the rate of growth, and result in earlier flowering. Warfield [26] reported that the low doses of γ-irradiation led to improvement in cell development of the *Saintpulia ionantha* plants, an increase in the growth rate, and earlier flowering. On the contrary, the high doses of gamma rays hindered the cell development, slowed growth, and delayed flowering, as reported by Radwan [27], who used gamma irradiation on *H. bracteatum* seeds that were subjected to eight different doses of gamma radiation (5–40 Gy). The results revealed that decreasing gamma dosages resulted in early flowering, whereas increasing gamma dosages

resulted in delayed flowering. Kumar and Mishra [28] mutated *Eclipta alba* seeds by different doses of gamma rays (100, 200, and 300 Gy). The results of the study showed that the lowest doses had a stimulatory influence on days to 50% flowering, but higher doses took greater number of days to 50% flowering compared with the control.

The number of flowers (inflorescences)/plant

It is shown in Tables 3 and 4 that three different doses of gamma irradiation (10, 20, and 40 Gy) significantly increased the number of flowers/plants in both generations by 120.66, 118.92, and 107.47, respectively, in MG1 and 126.85, 122.69, and 116.19, respectively, in MG2, with increments of 26.31, 24.49, and 12.51%, respectively, in MG1 and 20.36, 16.41, and 10.24%, respectively, in MG2. On the contrary, the lowest number of flowers/plant was recorded by a dose of 60 Gy (78.00) in MG1 and 50 and 60 Gy (81.78 and 85.41) in MG2, with a decrement of 18.34% in MG1 and 22.40 and 18.95%, respectively, in MG2 compared with the untreated plants. Several previous studies indicated that small doses of gamma irradiation caused an increase in the vegetative growth characteristics of many plants in many cases, whereas greater levels had negative effects [29]. The findings acquired are consistent with those provided by Minisi *et al.* [21], who used gamma rays on *M. laevis* treated with doses of 0, 2.5, 5, 7.5, 10, 12.5, 15, 17.5, and 20 Kr. The seeds

Figure 3



The plant abnormalities of *Gaillardia pulchella* as affected by different doses of gamma rays on leaf shape in MG1 and MG2 generations. (a) Control original leaf plants (1, loped; 2, dentate; and 3, serrulate); (b) 10 Gy (1, curried; 2, loped with biforked; and 3, deeply loped with large size in MG1 and MG2); (c) 20 Gy (1, deformed; 2, deeply lobed; 3, tribladed leaf; and 4, runcinate with colored midrib in MG1 and MG2); (d) 30 Gy (1, deformed; 2, biforked and 3, parted leaf in MG1 and MG2); (e) 40 Gy (Biforked in MG1 and MG2); (f) 50 Gy (1, simple biforked; 2, deformed with simple curvature; and 3, mightily biforked MG1 and MG2); (g) 60 Gy (1, triforked; 2, biforked; 3, deeply lobed; 4, serrated lobed; and 5, cuminate in MG1 and MG2); and (h) 60 Gy (doubly serrate with colored midrib in MG2).

were soaked for 12 h in water before treatments, whereas other seeds were used as dried. Low dose (2.5 Kr) of dry and wet seeds increased flowers number per branches. El-Khateeb *et al.* [19] found that two doses of 5 and 10 Gy shortened blooming time and increased number of flowers of *H. bracteatum* in MG1 and MG2. Nelka *et al.* [30] reported the same on *Jasminum officinale*.

The flower (inflorescences) diameter (cm)

The data in Tables 3 and 4 show that the *G. pulchella* L. plants treated with γ-rays caused a marked increase in flower diameter (cm) in the MG1 and MG2, compared with the untreated plants. It was found that doses of 10, 20, and 40 Gy of γ-irradiation gave statistically significant differences in the flower diameter by 7.20, 7.16, and 6.37 cm, respectively, in MG1 and 7.77, 7.68, and 6.73 cm, respectively, in MG2, with an increase of 31.38, 30.65, and 16.24%, respectively, in MG1 and 37.27, 35.68, and 18.90%, respectively, in MG2, compared with the control plants (5.48) in MG1 and (5.66) in MG2. On the contrary, the smallest flowers in diameter were produced at a high dose of 60 Gy in the MG1, with an estimated decrease of 20.25%, whereas at 50 and 60 Gy in the MG2, with an estimated decrease of 8.65 and 24.02%, respectively. However, the dose of 30 Gy in both generations did not give any clear difference in the diameter of the flower, whether by a decrease or increase. These findings are in agreement with the findings of Pallavi *et al.* [31] on *Zinnia elegans* (75, 100, and 125 Gy). In comparison with the control, larger doses of gamma radiation reduced the number of flowers and smaller floral diameters; Rifnas *et al.* [32] used gamma radiation at 0, 10, 30, 50, 70, 100, and 150 Gy on *chrysanthemum*, in comparison with the control; it was observed that high levels led to a reduced number of flowers and floral diameters. Eid *et al.* [33] reported on *H. bracteatum* seeds, which were given eight doses of gamma radiation (5–40 Gy). They stated that small levels of gamma radiation enhanced the number of flowers and the width of the flowers.

Plant abnormalities

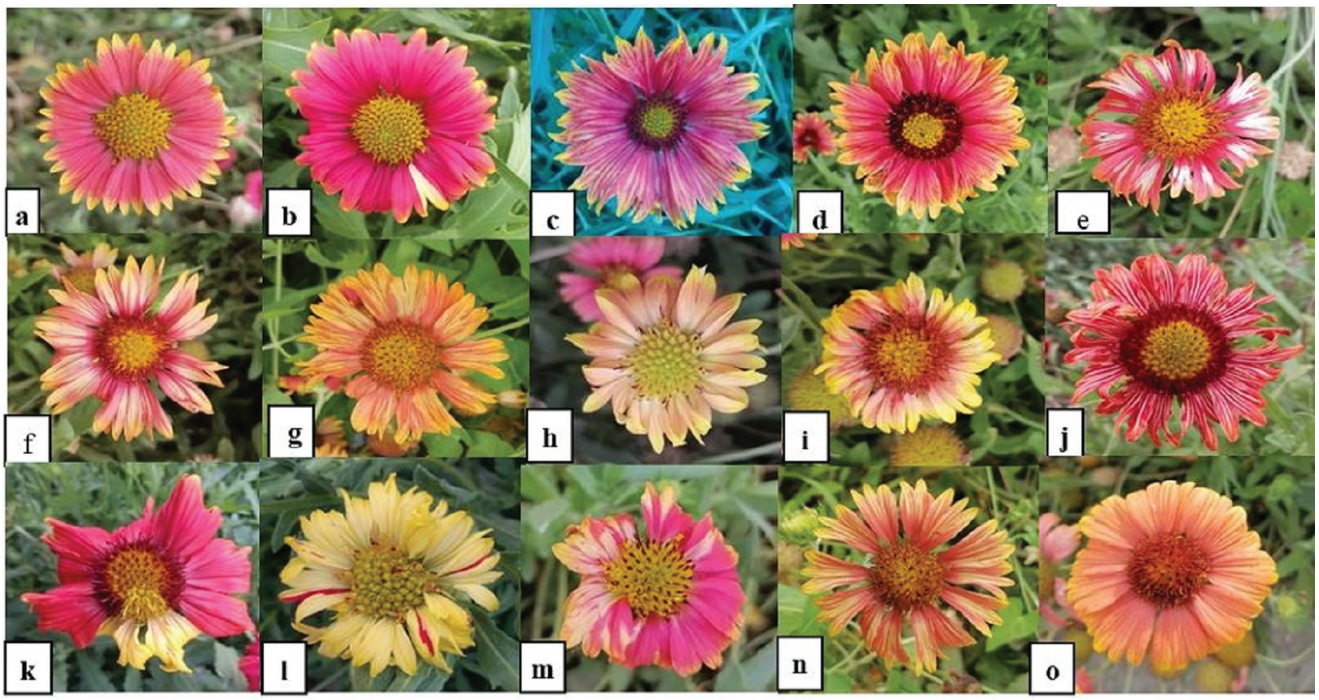
Leaf abnormalities

The results presented in Fig. 3 reveal that gamma irradiation-induced changes in the shape and structure of the leaves resulted in a considerable and obvious difference between the treated and untreated plants. Therefore, we selected and isolated the desired alterations by collecting the seeds of those plants that have the desired changes without cross-pollination and planting them in the following generation to develop new and enhanced traits through which a new Gaillardia strain might be produced. The study's findings revealed that using gamma rays had a large and effective effect on inducing various changes in the shape, structure, and size of the leaves. The results were in accordance with those reported by Mickaelsen *et al.* [34], who mentioned that the abnormalities observed in leaf shape may be attributed to disorders in phytochromes, mitotic inhibition, chromosomal aberrations, disturbances in the synthesis of DNA, and disrupted auxin synthesis. Moreover, Mostafa [35] found that variations and alterations in the structure or shape of the leaves of *Amaranthus* sp. and *Helianthus annuus* could be caused by chromosomal abnormalities and conferred as layer rearrangement as a result of influence of the mutagens. Yadav [36] studied the influence of gamma irradiation at different levels of 10, 15, 20, 25, 30, 35, 40, 45, and 50 Kr to induce mutants in *Canscora decurrens* Dalz. The results recorded many morphological variants, that is, leaf shape (ovate, obovate, and reniform) and leaf margin (crenate and serrate). Hapsari *et al.* [22] on *C. heyneana* employed gamma radiation at 10–50 Gy. They observed an apical split, stiffened, wrinkled, twisted, and variegated leaves.

Inflorescence abnormalities

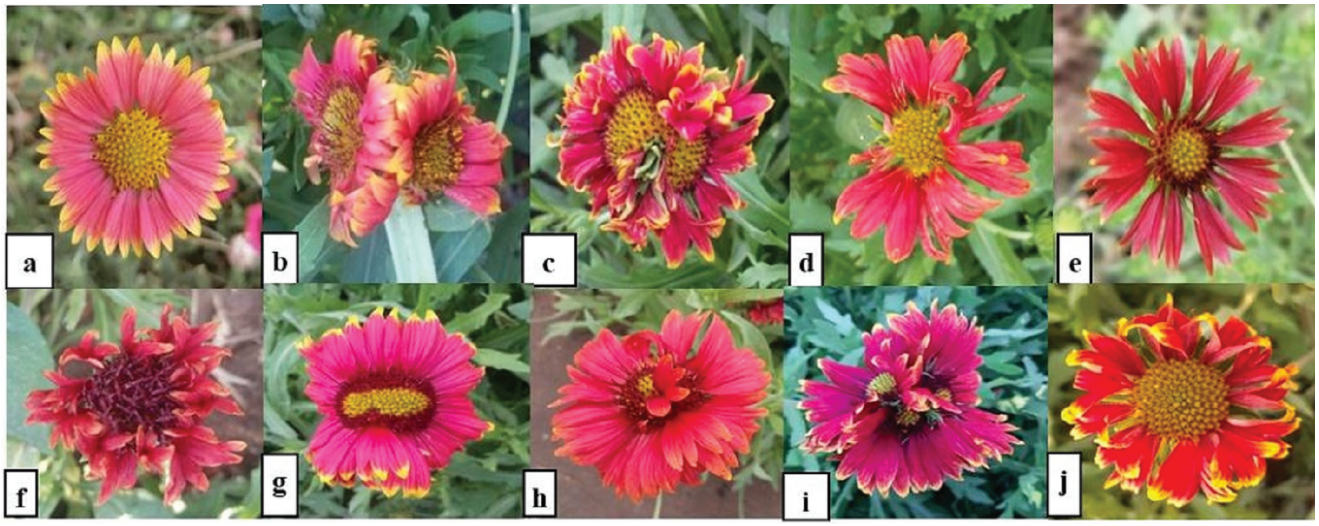
The results of the current work observed that all gamma doses induced variations in color, shape, and deformation in the inflorescences, compared with nonirradiated plants. Besides, the high doses of gamma rays (40, 50, and 60 Gy) recorded the largest number of mutants in the inflorescences (Figs 4 and 5). These variations in floral characteristics and the appearance of new flower shapes are the most desirable characterizations in ornamental plants in general and *G. pulchella* in particular. Moreover, these variations in floral characteristics may be attributed to a mutation in the biosynthetic pathway of regulatory or structural genes, which will generate a change in color and flower shape. According to Mato *et al.* [37], anthocyanin accumulation in different flower colors occurred during the blockage at the early and late stages of anthocyanin production. Datta [38] reported that variations in flower color could be ascribed to either qualitative or quantitative alterations in flower pigments as a result of γ-irradiation stimulated biosynthetic pathway changes. Many authors suggested that plants treated by γ-radiation can alter the vegetative characterization and flowering development in either a negative direction or a positive direction [39]. The range of

Figure 4



The color variations of inflorescences abnormalities of *Gaillardia pulchella* as affected by different doses of gamma rays on inflorescence color in MG1 and MG2 generations control original color, the outer half yellow, and the rest of the ray red. (b) 10 Gy chimeric mutation, yellow in part of one petal and the other red color in M1. (c) 20 Gy variegated radial flowers red with yellow in MG1 and MG2. (d) 40 Gy semidouble inflorescence, radial red flowers with orange interlacing in the upper part of the petals in MG1 and MG2. (e) 40 Gy variegated ray flowers in MG1 and MG2. (f) 40 Gy multicolored radial flowers with intertwining red with orange, and yellow in MG2. (g) 40 Gy orange radial flowers tinged with light red in MG1 and MG2. (h) 40 Gy radial yellow flowers tinged with light pink in MG2. (i) 50 Gy yellow radial flowers tinged with red at the bottom in MG2. (j) 60 Gy radial flowers variegated with longitudinal stripes in white with red colors in MG1. (k) 60 Gy chimeric mutation, four petals are yellow and the others are red in MG1. (l) 60 Gy chimeric mutation, some of the petals are stained red and others are pure yellow in MG1. (m) 60 Gy chimeric mutation, some of the petals are stained orange color in MG2. (n) 60 Gy light red and orange radial flowers intertwined together with slender petals in MG1 and MG2. (o) 60 Gy orange-colored radial flowers with broad petals in MG1 and MG2.

Figure 5

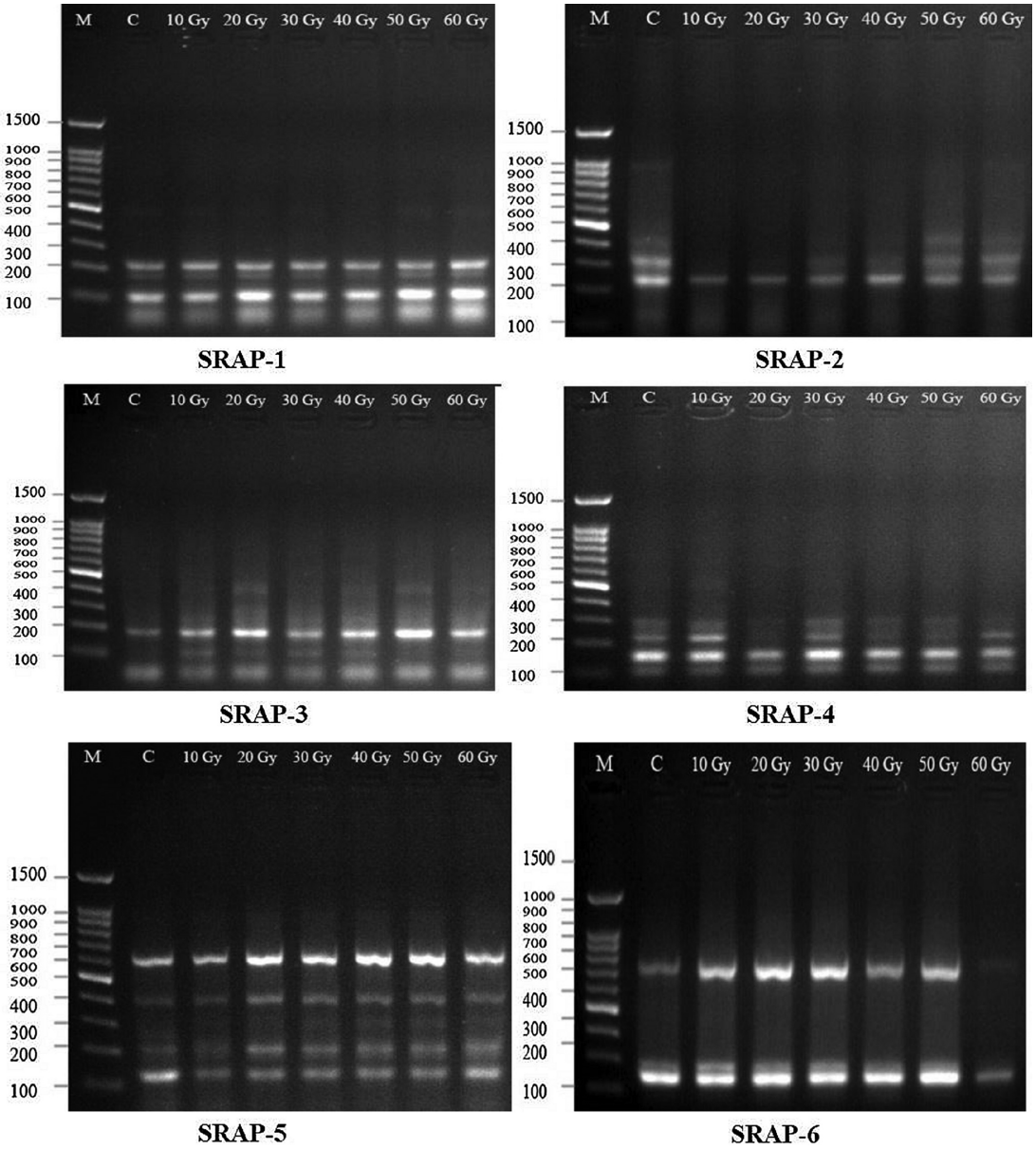


The deformation of inflorescences abnormalities of *Gaillardia pulchella* as affected by different doses of gamma rays on inflorescence in MG1 and MG2 generations control original color, the outer half yellow and the rest of the ray red. (b) 10 Gy two inflorescences on the same stalk in MG1. (c) 20 Gy fasciation inflorescence, two inflorescences on the same stalk in MG1 and MG2. (d) 30 Gy deformed radial flowers in MG1 and MG2. (e) 40 Gy slender ray flowers in MG1 and MG2. (f) 50 Gy deformed inflorescence, radial flowers are funnel-shaped and disc flowers are dark and sterile in MG1. (g) 60 Gy fasciation inflorescences in MG1 and MG2. (h) 60 Gy red radial flowers and the appearance of one petal in the disc flowers in MG2. (i) 60 Gy three inflorescences on the same stalk in MG1 and MG2. (j) 60 Gy coiled radial flowers with a bright red color and a yellow color at the endin MG2.

colors likely to be stimulated as a result of γ-rays varies with the flower color of the variety under study. It has been observed that the pink-colored varieties have the highest number of dominant genes responsible for altered flower color, consequently likely generating recessive mutations as identified by several authors [40]. Kaicker [41] observed that the appearance of new flower colors is caused by variations in the amount of anthocyanin pigments. Radiation-induced alterations in flower color could possibly be the result of altered pigment production pathways. In this regard, Eid *et al.* [33] reported that *H. bracteatum* seeds were given eight doses of gamma radiation (5–40 Gy). The findings revealed that various morphological changes in flower color were produced over two generations.

We reported our results on *G. pulchella* seed germination and vegetative and flowering growth. It can be stated that the low doses of γ-irradiation increased the plant height and number of branches (ramification), and the highest level of γ-rays reduced them in both MG1 and MG2. The stimulation effect of low doses can be interpreted as the exposure to low doses of γ-radiation at positively affecting the vegetative growth cycle and development, whereas the higher doses cause damage, which could be due to transferring and penetrating it into the cells. It reduces the process of photosynthesis and inhibits the process of biosynthesis of chlorophyll or carbon fixation, showing severe unrecovered physiological damage than that of low doses, leading to

Figure 6



SRAP analysis of *Gaillardia pulchella* plants treated with six different doses of gamma irradiation, using primers SRAP-1, SRAP-2, SRAP-3, SRAP-4, SRAP-5, and SRAP-6. Lane M: 100 bp DNA ladder; lane C: the control plant.

abnormalities in several cases. Gamma rays are widely used as ionizing radiation to improve plant growth and chemical constituents, as well as to induce genetic diversity and improve many useful traits in plants. This technique creates new gene combinations with higher mutation frequencies, some of which may be useful for their higher economic values or as a new trait, such as plant size, shape, color, and size of flowers, and time of flowering, and resistance to pathogens, leading to the creation of new variants. In our study, irradiation treatments with low gamma doses exhibited beneficial effects on growth and flowering traits in the first season. This effect increased, and it was more pronounced in the GM2, showing remarkable increases in most traits.

Sequence-related amplified polymorphism markers of plants mutant with gamma radiation

Six primers were used for identifying DNA polymorphism among *G. pulchella* plants mutated by gamma irradiation and the control (Fig. 6 and Table 5). A total of 32 loci, ranging from 100 to 930 bp, were scored. Twelve bands out of 32 (37.50%) loci were polymorphic, whereas 20 (62.50%) amplified fragments were monomorphic. The highest number of amplicons was produced by primers SRAP-2 and SRAP-5 (seven loci), followed by primers SRAP-3 and SRAP-4 (five fragments), whereas the lowest number of bands was generated by primers SRAP-1 and SRAP-6 (four bands) (Table 5). On the contrary, primer SRAP-2 displayed 71.43% polymorphism, followed by SRAP-3, which gave 60% polymorphism. However, no polymorphic bands were scored in primer SRAP-1; it gave only monomorphic bands (Table 5).

Cluster analysis

The genetic identity values among *G. pulchella* plants mutated by gamma rays and the control ranged from 0.69 to 0.96 (Table 6). The lowest genetic similarity was recorded between the control and 10 Gy (0.69%), whereas the highest genetic identity was scored between 10 and 20 Gy, 20 and 30 Gy, 30 and 40 Gy, and 50 and 60 Gy (96%) (Table 6). A dendrogram indicated three different groups. The first group (I) involved 10-, 20-, 30-, and 40-Gy mutants. The second group (II) included 50- and 60-Gy mutants. The third group (III) contained only the control, which refers to a higher genetic distance between the control and other mutants (Fig. 7). Therefore, the application of SRAP markers in ornamental breeding programs could facilitate the screening of new mutations. Previous reports have stated that gamma rays have the potential to create new *Curcuma alismatifolia* types with enhanced commercial characterization suitable for the flower industry. The use of molecular markers will help in the discovery of genetic diversity among mutated and unmutated plants, as well as the identification of plants with morphological properties [39]. The appearance or

Table 5 Sequence-related amplified polymorphism analysis of *Gaillardia pulchella* plants (MG2) mutated by gamma rays

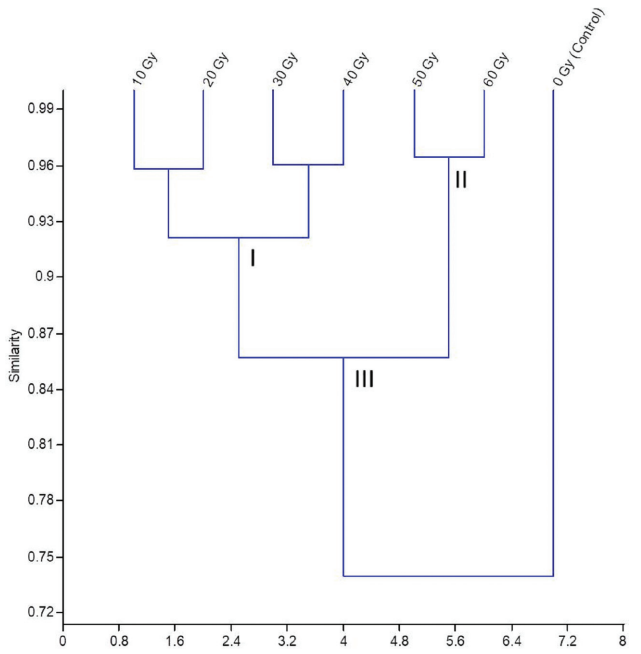
Primer name	Size range of the scorable bands (bp)	Total bands	No. of monomorphic bands	No. of polymorphic bands	% polymorphism	Unique markers	Molecular size of markers (bp)
SRAP-1	105–500	4	4	0	0	0	–
SRAP-2	100–930	7	2	5	71.43	+2	+133;+505
SRAP-3	113–421	5	2	3	60	–3	–224; –285;–421
SRAP-4	105–505	5	4	1	20	+1	+505
SRAP-5	112–610	7	5	2	28.57	+1	+500
SRAP-6	180–790	4	3	1	25	–1	–191
Total	10–930	32	20	12		8	
Mean		5.33	3.33	2.00		1.33	
%			62.50		37.50	25	

SRAP, sequence-related amplified polymorphism.

Table 6 Distance matrix depended on Jaccard similarity coefficients in *Gaillardia pulchella* plants mutated by gamma rays

Dose	0 Gy (control)	10 Gy	20 Gy	30 Gy	40 Gy	50 Gy	60 Gy
0 Gy (control)	1.00						
10 Gy	0.69	1.00					
20 Gy	0.71	0.96	1.00				
30 Gy	0.75	0.92	0.96	1.00			
40 Gy	0.72	0.88	0.92	0.96	1.00		
50 Gy	0.79	0.82	0.85	0.89	0.93	1.00	
60 Gy	0.77	0.79	0.82	0.86	0.89	0.96	1.00

Figure 7



Dendrogram of *Gaillardia pulchella* plants mutated by gamma irradiation based on Jaccard's similarity coefficients, compared with the control.

disappearance of loci after gamma rays is attributed to a change in the plant's DNA; molecular analyses have shown that gamma irradiation can cause DNA rearrangements in the plant genome [42]. Furthermore, the incidence of mutations varied greatly from one plant to another, depending on the species, variety, and the dose [43]. Thacker [6] discovered that gamma irradiation may induce some mutagens to cellular genes via the DNA repair strategy in cells. The treatment by mutagens may break the nuclear DNA, and during the DNA repair process, new mutations occur at random and are heritable. Plant breeders can choose advantageous mutants such as flower shapes, flower colors, and early flowering types by causing mutations in cytoplasmic organelles, resulting in chromosomal or genomic changes [8]. Radiation-induced mutation has effectively produced a significant number of new varieties in different seeds and ornamental plants and is regarded as a highly effective method for ornamental plant breeding [9].

Conclusion

This work highlights the genetic enhancement of the *G. pulchella* plant by gamma irradiation. The results showed that low doses (10 and 20 Gy) of gamma rays had significant effect on vegetative growth (plant height and the number of branches per plant) and flowering growth (early flowering, an increase in the number of flowers, and inflorescence diameter) in both MG1 and MG2, compared with the untreated plants. On the contrary, SRAP markers were used to confirm the existence of genetic diversity at the genomic DNA level among populations treated with gamma rays and control, depending on the dose. Therefore, SRAP markers are considered an important tool for detecting the mutagenic effects of gamma rays. Moreover, it will help to discriminate the populations showing variations in morphological and floral characteristics.

Acknowledgements

Source(s) of support: This work was supported by National Research Centre, Dokki, Giza, Egypt.

Presentation at a meeting: National Research Center.

Author contributions: all authors designed the work, carried out the practical experiments, and analyzed and interpreted the data. All authors contributed toward writing the manuscript and approved the final manuscript.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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