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# Breeding mutation by gamma radiation on $M_3$ plants for improvement of morphological and physical characters in *Gaillardia pulchella* Foug. Plants

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**Abstract**
Gamma radiation is an effective mutational breeding method for improving various growth and floral characteristics of ornamental plants. This Experiment was undertaken with an objective to investigate the effect of gamma irradiation on the growth and floral characteristics of *G. pulchella* plants during the third generation ($M_3$). Irradiation treatments were performed on the seeds in the first generation only, Gamma-1 type cobalt60 was used to expose seed plants to different dosages such as 0, 10, 20, 30, 40, 50 and 60Gy. The results indicated that control plants gave the highest values of seed germination; meanwhile, the lowest value was recorded with 60 Gy. The tallest plants, greatest leaves number, biggest stems, longest roots, early flowering, and largest flowers number were achieved with the doses of 10, 20, and 40 Gy over the control. In contrast, with increasing the radiation level to 50 and 60 Gy had a negative effect, and decreased these characters to the lowest values, but increased the number of days elapsed to flowering. All the investigated characters exhibited significant positive correlations, except for number of days from planting to flowering, which was significantly negatively correlated with all characters. All flowering characters correlated with each other in a medium to high significant correlation. Using gamma increased relative water content, but decreased electrolyte leakage compared to the control. All gamma doses decreased membrane stability percentage; in except for the smallest dose (10 Gy). Using 10 and 20 Gy gave the highest values of number of disc flowers rows, diameter of flowering bud, and receptacle.

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**Keywords:** *Gaillardia pulchella*, gamma rays, improvement, morphological characters, anatomical structure, (RWC), (EL), and (MSI)

**INTRODUCTION:**

*Gaillardia pulchella* Foug. (Asteraceae) is commonly known as American blankets, Indian blanket flower, or Sundance. They are flowering plants native to the Central United States and are widely cultivated in Egypt for their ornamental value. Genus Gaillardia includes about 23 species, but only three of which are commonly known, these include *G. pulchella*, which is an annual plant, while *G. grandiflora* and *G. aristata* are perennial plants. Now their cultivation had been expanded throughout the world because of its similarity to *Chrysanthemum* and the possibility of year-round cultivation (Jadhav *et al.*, 2022). The inflorescences are single or double flower heads that have bands of colors, maroon, red, or orange; the flowers bloom April to September (Kale, 2002). The leaves are alternate and hairy; with smooth, toothed or lobed edges. Fruit has a pappus of scales. The plants are excellent for borders or mixed beds, house gardens, containers, streets, and tolerate dry conditions (Agale *et al.*, 2017). The sesquiterpene lactones are characteristic compounds among the numerous secondary metabolites found in the Asteraceae family and in the *Gaillardia* species, and they can be considered chemotaxonomic markers, while some flavonoids are probably typical for them. It has also been reported that the extracts of the *Gaillardia sp*. possess antiparasitic, antitumoral, and cytotoxic activities (Yu *et al.*, 1988). The antihypoxic and anti-ischemic properties of some extracts obtained from the inflorescences of *G. pulchella* reduced the level of ischemic damage to the brain and also reduced tissue hypoxia due to restoration of mitochondrial functions (Harimaya and Inayama, 1990).

Gamma irradiation (Cobalt-60) is one of the main physical mutagens for mutation studies in plants, it can be interact with atoms and molecules, thus producing free radicals in cells which are responsible for modifying important components of plant cells that affect morphology, anatomy, biochemistry, and physiology of plants, depending on the irradiation dosage (Wi *et al.*, 2005). Irradiation technology could be used for improving the quality of medicinally valuable plants, including their biomass production (Akshatha *et al.* 2013). Gamma rays belong to ionizing radiation and interact to atoms or molecules to produce free radicals in cells, which affects plant cellular structure and metabolism, e.g., the dilation of thylakoid membranes, alteration in photosynthesis, modulation of the antioxidative system, and accumulation of phenolic compounds (Wi *et al.*, 2005, and Wang *et al.*, 2016). Gamma rays were employed to developed 64% of the radiation induced mutant varieties, followed by 22% by X-rays (IAEA, 2015) gamma rays is an energetic form of electromagnetic radiations are recognized as the widely used mutagen for their simple application, good penetration, reproducibility and high mutation frequency (Chahal and Gosal, 2002) gamma rays having short wavelength with high penetrable power, interact with atoms or molecules to produce free radicals in the cells. Among the physical mutagens, gamma ray stands first in their effectiveness in the induction of mutation in crop plants (Jan *et al.* 2011 and Verma *et al.* 2012). Physical mutagens have been used by many studies to improve ornamental plants, (Khattab and Hegazi 2015) on *Matthiola Dimorphotheca* and *Dianthus,* (El-Khateeb *et al.*, 2016) on *Philodendron*, (Pallavi *et al.*, 2017) on *Zinnia*, (Sarhan *et al.*, 2019) on *Tagetes,* (Chen *et al.*, 2020) on *Chrysanthemum,* (Elmenbawy *et al.*, 2020) on *Calendula*, (Eid, 2021) on *Helichrysum,* (Hartati *et al.*, 2021) on orchid, (Al-Haidari and Al-Tamimi, 2023) on *Catharanthus*, and (Fayed, 2023) on *Borgo*. This study aimed to investigate the effect of gamma irradiation on improving vegetative growth, flowering, and chemical characters in *G. pulchella* in the third generation.

**Materials and methods:**

**Plant materials**

The seeds of *G. pulchella* (local variety) were obtained from a bred strain in The Ornamental Horticulture Department of Agriculture Cairo University Egypt. A field experiment was conducted in this location during the third generation ($M_3$) of 2021/2022.

**Treatment of seeds**

Irradiation treatments were performed on the seeds in the first generation ($M_1$) only. The seeds of *G. pulchella* (batches of 500 seeds for each treatment) were pre-soaked in distilled water for 1 hour before being irradiated with gamma radiation at Atomic Energy Commission-united irradiation-Gamma, Nasr city, Egypt, at the doses of (0.0, 10, 20, 30, 40, 50 and 60 Gy). The control seeds were kept without irradiation. Gamma-irradiation doses were done using Indian gamma cell (Ge 4000 A), type cobalt 60 (Co60) and a dose rate at 1.107 KGy/h.

**Preparing seedlings, transplantation, and growth conditions for the selected mutants of $M_3$ (2021/2022)**

The mass selection of seeds for some selected traits such as: early flowering plants, plants with strong vegetative and flowering growth, plants with variations in flower color, shape and size, and short-growing plants that may be used as potted plants in 2019/2020 for $M_1$ plants was done in May 2020, where plants that survived in each treatment were evaluated, selected, and selfed in order to obtain the second mutative generation 2020/2021 ($M_2$) seeds. Observations were taken during the vegetative growth and flowering periods. In order to prevent cross- pollination between plants and some of them, whether by wind or insects, we used a bag of paper for the flower buds before opening in order to preserve the selected characters and to grow $M_2$ generation (seedlings) plants. Field selections were done for selected traits in the $M_2$ to obtain the third mutative generation seeds ($M_3$) 2021/2022, according to Sinhamahapatra and Rakshit (1990). The selected seeds for $M_3$ were sown in plastic trays filled with a mixture of peat moss, loam, and sand (1:1:1 by volume) Fig. (2) on 5 October 2021) to produce seedling. After 10 days of sowing seeds began germination, and 45 days after sowing, uniform seedlings (8-10 cm in 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 row (two plants/hill), every plot (3.5 x 1.8 m) contained 21 hills /plot.

**Agricultural practices:** All the recommended cultural practices namely, irrigation and fertilization, were carried out during the plant's growth and flowering period. The fertilizers were supplied for each plot as recommended, using Kristalon mineral fertilizer (N:P:K) at 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 needed amount of water, and weeding was carried out as the soil needed.

**Soil analysis:**

Soil analysis revealed that, particle size distribution (%) was: sand: 25.6, silt: 27.3 and clay: 37.5 (texture: clay loam), pH: 7.2, EC ds.m-1: 0.93.

**Data recorded**

The following data were recorded on *G. pulchella* plants that grew until the flower opening reached 50%, that is, about a month after the flowers started to appear for each treatment:

**(a)** Seed germination (%) was measured using the Petri dish method: 100 seeds were placed on filter paper in a Petri dish for each treatment, the filter paper was thoroughly moistened with distilled water, and the Petri dishes were then covered with Parafilm at laboratory temperature. The moisture content of the filter paper was checked daily until the seeds began to germinate within five to ten days (Fig. 1). The germination percentage was estimated using the following equation:

$$\textbf{Germination (\%)} = \frac{\textbf{No.of seeds germinated}}{\textbf{Total No.of seeds sown for germination}} \times \textbf{100}$$

**(b)** Vegetative characters [plant height (cm), No. of main branches/plant, leaves number/plant, stem diameter, and root length]. **(c)** Flowering characters [No. of days from planting to flowering (DPF), and number, diameter and stalk length of inflorescences/plant]; **d.** Correlation coefficients among of vegetative, and floral characters. **(e)** Water status in leaves [relative water content (RWC), electrolyte leakage (EL), and membrane stability index (MSI)]. **(f)** Inflorescences mutants. **(g)** Anatomical structure of the leaves and inflorescences [Samples of plants (leaves and flowers) were fixed and killed for at least 48 hours in F.A.A. (10 ml formalin, 5 ml glacial acetic acid, 50 ml ethyl alcohol 95% and 35 ml distilled water), washed in 50% ethanol, dehydrated in normal butyl alcohol series and embedded in paraffin wax, according to Sass (1951). Sections of leaves and flowers were cut to a thickness of 20 microns and stained with safranin light green combination and mounted in Canada balsam, according to Nassar and El-Sahar (1998). The slides were microscopically examined; counts and measurements (µm) of the different tissues (leaves and flowers) were taken and calculated using a micrometer eye piece; were pictured using light microscope with camera model Leica ICC50 HD at Faculty of Agriculture Research Park, Cairo University].

**Statistical analysis:**

Statistical analysis was conducted using COSTAT software; a randomized complete block design was used, with three replicates for each treatment and 10 plants in each replicate. The results of the four trials were statistically analyzed using Snedecor and Cochran's (1980), and the means were separated using Duncan (1980) multiple range tests and compared using the L.S.D test at 0.05 probability.

**Results and discussion:**

**Seed germination (%):** Evidenced by the results in Table (1) and Fig. (1) that exposing the seeds to gamma radiation had a negative effect on seed germination (%), all doses of gamma radiation decreased the germination percentage when compared to the control that recorded the maximum germination percentage (90.03), the low gamma doses of 10 and 20 Gy increased the germination percentages to (82.71% and 81.03%), compared to the high dose of 60 Gy that recorded the lowest germination percentage of (54.54%). In this regard, Melki and Marouani (2010) suggested that the decrease in seed germination caused by high dosages may be attributed to cellular disorders, including chromosomes damage, seed tissue injury, a decrease in seed moisture content, degradation of meristematic cells, and mitotic delay. In contrast, low 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, as reported by Abd El-Hady *et al*. (2008). The results are in agreement with those reported by Radwan (2017) on *Helichrysum bracteatum*, they subjected seeds to eight different doses of radiation (5–40 Gy), and they revealed that all gamma doses reduced seeds germination compared to the control. Chedeo and Wamaedeesa (2021) mutated garden balsam seeds with gamma doses of 0, 50, 100, 200, and 300 Gy. All gamma doses decreased seed germination, and El-Khateeb *et al*. (2023) on *Gaillardia pulchella***,** they recorded a reduction in seed germination compared to the control, when irradiating the seeds with six doses from (10 – 60 Gy).

**Table (1) Effect of gamma rays treatments on seed germination, plant height, No. of main branches/plant, No. of leaves/plant and stem diameter of *G. pulchella* plant, during the $M_3$ generation (2021/2022)**.

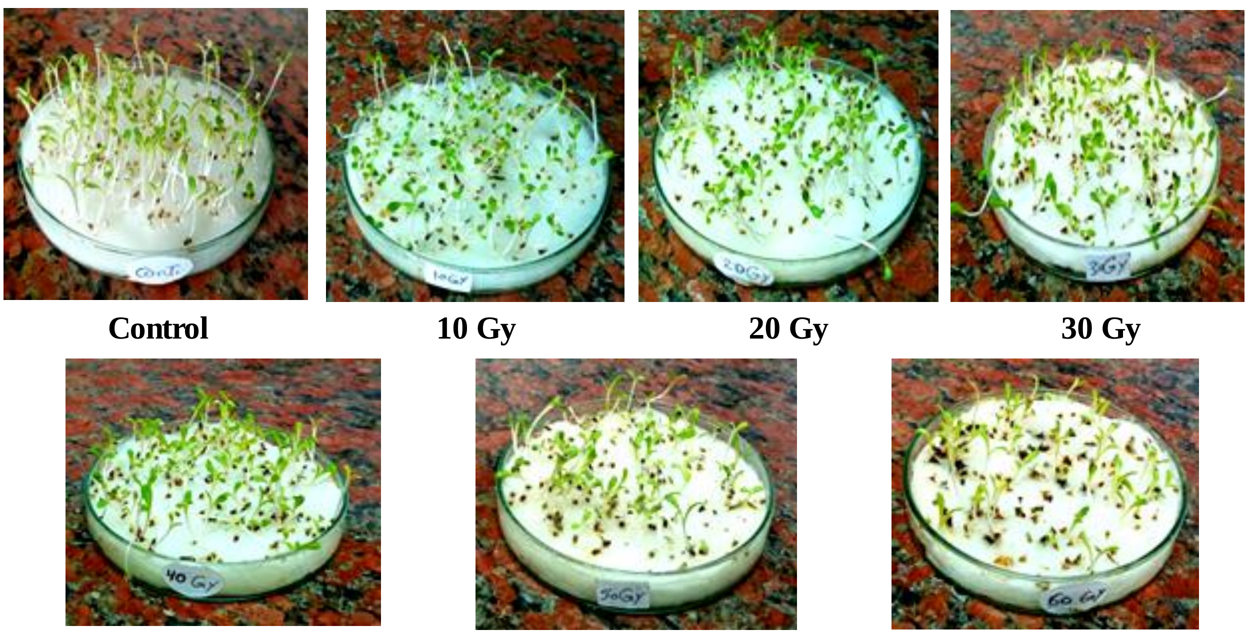
| Treatments | Seed germination | Plant | No. of main | Number of | Stem |
|---|---|---|---|---|---|
| **Control** | 90.03 a | 114.90 c | 15.33 bc | 520.33 d | 1.76 bc |
| **10 Gy** | 82.71 b | 127.39 a | 18.30 a | 630.30 a | 2.40 a |
| **20 Gy** | 81.03 b | 126.28 a | 16.70 ab | 610.09 b | 1.90 b |
| **30 Gy** | 72.03 d | 117.80 b | 13.06 cd | 510.33 d | 1.60 c |
| **40 Gy** | 76.16 c | 119.35 b | 15.03 bc | 570.85 c | 1.80 b |
| **50 Gy** | 64.10 e | 97.65 d | 12.60 cd | 450.60 e | 1.35 d |
| **60 Gy** | 54.54 f | 93.66 e | 10.37 d | 380.25 f | 1.40 d |

**b. Vegetative characters**

According to the data presented in Table (1) the longest plants (127.39, 126.28, 117.80 and 119.35 cm) were observed with gamma doses of 10, 20, 30 and 40 Gy respectively, compared to (114.90 cm) for the untreated plants, with an increment of (10.87%, 9.90%, 2.52% and 3.87%), respectively, compared to the control. While, the shortest plants were produced with gamma doses of 50 and 60 Gy (97.65 and 93.66 cm) with a decrement of (15.01% and 18.48%), respectively, compared to the control.

Treating plants with gamma radiation at 10 Gy increased the formation of branches/plant, giving 18.30 branches, by increment of 19.37%, but the highest gamma dose of 60 Gy resulted in the lowest number of branches by 10.37 branches, with a reduction of 32.35%, when compared to (15.33 branches) for the untreated plants.

Also, the results showed that, the gamma doses of 10 as well as 20 then 40 Gy increased the formation of leaves to the highest values of (630.30, 610.09 and 570.85 leaves) by increment % (21.13%, 17.25% and 9.70%), respectively, compared to (520.33 leaves) for the control, and with increasing the radiation level to 50 and 60 Gy had a negative effect, and gave the minimum number of leaves (450.60 and 380.25 leaves) by decrement % (13.40% and 26.92%), respectively. As for the stem diameter, in comparison with the control, it was found that the low dose of 10 Gy induced a significant increase in stem diameter, and it gave the biggest diameter (2.40 cm) by increment % (36.36%), compared to the control. In contrast, the smallest diameter of stem was achieved with the high doses of 50 and 60 Gy, giving values of (1.35 and 1.40 cm) with a reduction of (23.29% and 20.45%), respectively, compared to the control that gave (1.76 cm). Concerning the data in Table (2) it indicated that using gamma doses at 10, 20, and 40 Gy produced the longest roots, giving values of (22.30, 20.41, and 19.67 cm) by increment % (25.98%, 15.31% and 11.12%), respectively, compared to the control. Meanwhile, the dose of 60 Gy produced the shortest roots, giving the value of (16.85 cm) by decrement % (4.80%).



**Control** **10 Gy** **20 Gy** **30 Gy**

**40 Gy** **50 Gy** **60 Gy**

**Fig. 1. Showing the seed germination (%) of *G. pulchella* in Petri dishes as affected by different doses of gamma rays in $M_3$.**

**Control** **10 Gy** **20 Gy** **30 Gy** **40 Gy** **50 Gy** **60 Gy**

**Fig. 2. Showing the seedlings of *G. pulchella* in plastic trays as affected by different doses of gamma rays in $M_3$.**

In this concern, Pitirmovae (1979) stated that the low doses of gamma rays increased vegetative growth, and attributed this to cell division or cell elongation, as well as changes in metabolic processes that affect phytohormone and nucleic acid synthesis. Chakravarty and Sen (2001) on the *Scilla indica* plant stated that the stimulatory effects of low gamma doses by enhancing growth rate due to speeding cell division. In contrast, the high doses causes a marked direct or indirect damages in morphology and physiology plant, and the exposure to gamma at high doses negatively affected the vegetative growth cycle and development, and show direct damage due to transferring and penetrating it to the cells and its content of DNA, leading to cell death or damage, which in several cases produce abnormalities in most traits, as reported by Jan *et al.* (2013). Joshi *et al.* (2011) reported that the high doses of gamma radiation decreased plant growth, and attributed this to the physiological injury to tissues and cells, resulting in a switch from normal to stunted growth, due to rise in growth inhibitors, a reduction in auxin concentrations or auxin synthesis inhibition, alterations in ascorbic acid concentration, and physiological and biochemical abnormalities of metabolites, causing the stunted growth, as identified by Roychowdhury and Tah (2011).

**Table (2) Effect of gamma rays treatments on average root length, No. of days to flowering, number, and diameter of inflorescences/ plant, and inflorescences stalk length of *G. pulchella* plant, during the $M_3$ generation (2021/2022).**

| | Average root | No. of days to | No. of inflorescences | Inflorescences | Inflorescences |
|---|---|---|---|---|---|
| Control | 17.70 cd | 177.20 c | 102.36 d | 6.20 c | 19.70 c |
| 10 Gy | 22.30 a | 163.03 e | 127.76 a | 7.90 a | 22.39 b |
| 20 Gy | 20.41 b | 165.66 de | 119.80 b | 7.96 a | 24.33 a |
| 30 Gy | 17.90 cd | 176.25 c | 98.36 e | 6.30 c | 18.60 cd |
| 40 Gy | 19.67 b | 169.15 d | 109.90 c | 7.03 b | 20.50 c |
| 50 Gy | 18.30 c | 187.50 b | 89.25 f | 5.40 e | 17.71 d |
| 60 Gy | 16.85 d | 193.70 a | 79.30 g | 5.60 d | 15.09 e |

These results are similar to those found by Omar (2016) on *Lathyrus odoratus*, he found that the low level 10 Gy of gamma enhanced the plant height and branches number. Radwan (2017) on *Helichrysum bracteatum*, found that using gamma 10 Gy induced a significant increase in plant height compared to the control. Bosila *et al.* (2020) on *Chrysanthemum morifolium*, they mentioned that the low doses of 0, 5, 10 and 20 Gy increased plant height. Ghosh *et al.* (2020) observed that using the low gamma doses of 10, 15, 20 and 25 Gy, increased the *Jasminum grandiflorum* height and number of branches. Rifnas *et al.* (2020) discovered that using the low doses of 0, 17, 18, 19, 20 and 21 Gy increased *Allamanda cathartica* plant height. Hartati *et al.* (2021) reported that exposing the seeds of *Vanda orchid* to gamma doses at 10 and 20 Gy induced a significant increases in the morphological characteristics of the plant compared to the control. Hajizadeh *et al.* (2022) found that subjecting lilium bulbs to gamma dose of 50 Gy had a negative effect on the vegetative growth characteristics of the plant compared to the control, and Radwan (2023) found that the most vegetative growth characters increased with the low doses of

10 and 20 Gy, while the large doses of 50 and 60 Gy decreased them on *Gypsophila elegans* plant.

**c. Flowering characters**

Table (2) revealed that, the doses of 10 followed by 20 as well as 40 Gy, caused early flowering, shortened the vegetative growth phase, the average number of days from the sowing seeds until the beginning of flowering was 163.03, 165.66 and 169.15 days, respectively, compared to 177.20 day for the control. On the other hand, the doses of 50 and 60 Gy prolonged the vegetative growth phase to 187.50 and 193.70 day, respectively, compared to the control. Concerning the data of number, diameter and stalk length of the inflorescences, it was found that treating plants with the low doses of 10 and 20 Gy significantly increased these characters to the highest values 127.76 and 119.80 inflorescences and 7.90 and 7.96 cm, 22.39 and 24.33 cm for inflorescence stalk length by increment of 24.81% and 17.03%, 27.71% and 28.38%, 13.65% and 23.50%, respectively, but with increasing the radiation to 50 and 60 Gy decreased them to the lowest values 89.25 and 79.30 inflorescences, 5.40 and 5.60 cm, 17.71 and 15.09 cm, respectively, by decrement of 12.80, 22.52, 12.90%, 9.67, 10.10 and 23.40% respectively.

In this regard, Ismael and Mahmoud (2015) stated that low doses of gamma rays enhanced the initiation of flowering because several biosynthesis pathways are thought to be changed, both of which are directly and indirectly related to flowering physiology. Kumari and Kumar (2015) on gladiolus plant, reported that the stimulatory responses of low doses of radiation were thought to be related to early variations in axillary bud development and changes in the initial pace of floral differentiation or increased, which could be attributable to a minor increase in photosynthetic activities induced by irradiation or because of processes or inhibition of mitotic and chromosomal alterations or disruption; 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 (1973) reported that the low doses of gamma 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. Hasbullah *et al.* (2012) on *Gerbera jamesonii*, suggested that the substantial effect of low gamma rays on early flowering may be attributed to increase hormone synthesis and therefore on bud production, and Shukla *et al.* (2018) on *Gladiolus grandiflorus* plant, observed that, low doses resulted in early flowering, with increasing gamma doses led to a delay it, and stated that the effect of gamma doses may be due to many biosynthesis pathways were considered to be changed as a result of mutagenesis actions, and these biosynthesis processes are directly and indirectly related with flowering physiology. The results obtained are in consistent with Abdel Mageed *et al.* (2016) on *Lathyrus odoratus*, they mentioned that using the low dose of 10 Gy enhanced the formation of flowers. Bhusari *et al.* (2017) found that low doses improved flowering parameters on *Tagetes erecta*. Sabaghi *et al.* (2018)

on some carnation cultivars, irradiated seeds with gamma rays at 0, 15, 25, 35, 45 and 55 Gy and they noticed that the flowers number, was decreased as the dosage was increased. Bosila *et al*. (2020) on *Chrysanthemum morifolium*, they found that the doses of 5 and 10 Gy significantly increased the number and diameter of flowers. El-Khateeb *et al*. (2022) on *Borgo officinalis*, and Radwan (2023) on *Gypsophila elegans*, who noticed that the number, diameter and stalk length of flowers increased with the doses of 10 and 20 Gy, while the doses of 50 and 60 Gy reduced them to the minimum values.

**d. Correlation coefficients among of some vegetative and floral morphological characters:**

Data in Table (3) indicated that all characters exhibited significant positive correlations, except for the number of days to flowering (DPF), which was negatively significantly correlated with all characters. The correlation was medium significant (r = 50 to 70) to highly significant (r = 70 to 90) between seed germination (%) and all vegetative and flowering characters, except for the average root length, which was low correlated (r > 50). The correlation was medium (r = 50 to 70) to highly significant (r = 70 to 90) between plant height and all vegetative and flowering characters, this relationship was very high (r ≥ 90) between plant height and each of the number of leaves and branches/plant. All flowering characters correlated with each other in a medium significant (r = 50 to 70) to a high significant (r = 70 to 90) correlation relationship.

**Table (3) Correlation coefficients among of some vegetative and floral characters as affected by gamma radiation**

| | SG (%) | PH (cm) | NB/p | NL/p | SD (cm) | ARL (cm) | DPF | NI/p | ISL (cm) | ID (cm) |
|---|---|---|---|---|---|---|---|---|---|---|
| **SG (%)** | | | | | | | | | | |
| **PH (cm)** | 80.16** | | | | | | | | | |
| **NB/p** | 74.69** | 82.46** | | | | | | | | |
| **NL/p** | 79.30** | 94.81** | 87.55** | | | | | | | |
| **SD (cm)** | 68.75** | 81.89** | 83.03** | 85.52** | | | | | | |
| **ARL (cm)** | 44.09* | 70.83** | 78.95** | 73.41** | 76.38** | | | | | |
| **DPF** | -76.64** | -93.17** | -80.48** | -97.25** | -82.63** | -65.01** | | | | |
| **NI/p** | 76.57** | 93.96** | 87.28** | 97.41** | 90.43** | 82.41** | -93.57** | | | |
| **ISL (cm)** | 75.40** | 87.35** | 78.65** | 90.62** | 72.23** | 73.36** | -87.39** | 90.90** | | |
| **ID (cm)** | 62.59** | 89.11** | 77.72** | 92.41** | 85.10** | 72.49** | -92.01** | 93.86** | 86.63** | |

*****Significant at **P < 0.05**, ******Significant at **P < 0.01**, **SG (%)** = Seed germination (%), **PH (cm)**= Plant height (cm), **NB/p** = No. of branches/plant**, NL/p** =No. of leaves/plant**, SD (cm)**= Stem diameter (cm), **ARL (cm)**=Average root length (cm), **DPF** =No. of days to flowering, **NI/p** =No. of inflorescences / plant, **ISL (cm)**= Inflorescence's stalk length (cm) and **ID (cm)**= Inflorescence's diameter (cm).

**e. Physical characters (Water status in leaves)**

**1- Relative water content (RWC):**

Regarding the results of relative water content (RWC) Table (4) showed a significant increase with all gamma doses compared to the control, and the lowest RWC percentage (69.30%) was recorded with the dose of 50 Gy. On contrast, the highest RWC percentage 77.92% was recorded with the dose of 10 Gy, compared to (64.44%) for the control. The results indicated that all treatments of radiation were able to keep RWC above 60%. In this regard. Vinodhana and Ganesamurthy (2010) stated that, a critical reduction of (RWC) fewer than 50% could result in tissue death, and maintaining a relatively high rate of (RWC) when exposing the plants to stress conditions that affect growth, inferred that these the plants have a high ability to withstand and resist the stress, especially a lack of water, and reported that radiation treatment is a factor affecting the growth of plants and may sometimes represent stress on plants, especially at high doses.

In this respect, Akhtar (2015) investigated the effect of gamma radiation on *Solanum lycopersicum* plant with doses of 0, 5, 10, 15, 20, 25, 30, 35, 40 and 45 Kr, he reported that the doses of 5 and 10 Kr gave the greatest RWC (%), whereas the control gave the lowest RWC (%), and he stated that, plants treated with gamma radiation have a greater ability to absorb water when transpiration rates are higher in heat stress conditions than the plants not treated with gamma radiation, and the plants were exposed to low doses of gamma may be produced a number of heat stable proteins linked to stress tolerance, and Nikièma *et al*. (2020) suggested that the low gamma doses result in increases in RWC, the mutants with sufficient water in their leaves age more slowly; the plants with high percentage of RWC can reduce the impacts of drought during the growth stage, when the seeds of the *Sorghum bicolor* plant were irradiated with doses gamma at 200, 300 and 400 Gy, they were found to be more resistant to radiation.

**Table (4) Effect of gamma rays treatments on relative water content RWC (%), electrolyte leakage EL (%) and membrane stability index MSI (%) of *G. pulchella* plant, during the $M_3$ generation (2021/2022)**.

| Treatments | Relative water content | Electrolyte leakage EL | Membrane stability index |
|---|---|---|---|
| **Control** | 64.44 d | 24.57 a | 83.64 a |
| **10 Gy** | 77.92 a | 16.97 d | 81.92 ab |
| **20 Gy** | 75.95 ab | 20.71 bc | 79.88 cd |
| **30 Gy** | 73.33 b | 18.51 cd | 81.48 bc |
| **40 Gy** | 76.77 a | 17.26 d | 80.56 bcd |
| **50 Gy** | 69.30 c | 21.30 bc | 79.35 d |
| **60 Gy** | 69.33 c | 22.42 ab | 78.63 d |

**2- Electrolyte leakage (EL):**

Using gamma doses at 10, 40, 30, 20 and 50 Gy significantly decreased the membrane electrolyte leakage (EL), as shown in Table (4), which produced the lowest values of 16.97%, 17.26%, 18.51%, 20.71% and 21.30 %, respectively, compared to 24.57% for the control. Whilst, the dose of 60 Gy produced the highest value of

22.42% compared to the others doses. In this respect, Aladjadjiyan (2002) reported that the number of free ions in the plants extract reduced when the gamma radiation doses are increased, and therefore its electro conductivity reduced. Cojocaru *et al.* (2005) suggested that hypothesis for the increase in electrical conductivity caused by high-doses of gamma irradiation was metal ions activated lipid peroxidation, causing phospholipid membrane damage. Furthermore, gamma radiation may be cause a decrease the electrolyte leakage; this reduction may be due to cell membrane obstruction produced by aggregation and denaturation of intracellular biomacromolecules. These results are in good harmony with Hajizadeh *et al.* (2022) irradiated the bulbs of lily cut flowers with gamma doses at control, 10, 20, 30, 40 and 50 Gy, they showed that the electrolyte leakage significantly reduced after irradiation treatments.

**3- Membrane stability index (MSI)**:

The membrane stability index of leaves showed a trend toward decreasing with increasing the dose of radiation, as presented in Table (4), it was found that treating the plants with gamma radiation induced significant decreases in (MSI) with all gamma doses except for the dose of 10 Gy, which did not differ significantly from control and gave the largest percentage of 81.92% compared to other doses of radiation. Whilst, the smallest percentages of 79.35% and 78.63% were achieved with the doses of 50 and 60 Gy, respectively. It could be suggested that the reduction in the stability of the cell membrane when exposed to radiation may be attributed to damage of the membrane and electrolytes have leaked from it. It appears to be due to ion reflux and cell wall breakdown.

**f. The inflorescences mutants during the $M_3$ generation:** The inflorescences' mutants pictured in Fig. (3) illustrated that utilizing gamma doses induced many abnormalities and variants in the shape and color of the ray flowers, like, chimeric mutation, variegated radial flowers red with yellow, dark maroon radial flowers coiled from the bottom, slender ray flowers**,** fasciation inflorescence**,** multicolored radial flowers**,** funnel-shaped coiled petals, red radial flowers with acute tips, and three inflorescences on the same stalk**.** Besides, the largest number of these inflorescences' mutants was achieved with the doses of 40, 50 and 60 Gy. These mutants 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. Datta (1990) 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. Kaicker (1990) 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. 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 Dowrick and Bayoui (1996) on chrysanthemum plant. Mato e*t al.* (2000) on *Dianthus caryophyllus* plant stated that anthocyanin accumulation in different flower colors occurred during the blockage at the early and late stages of anthocyanin production. In this regard, Kaur *et al*. (2017) irradiated *Calendula officinalis* seeds with gamma radiation at 20, 40, 60, 80, and 100 Gy. They showed in comparison to the control, all doses induced many mutants in color, formation and shape of flowers. Li *et al*. (2022) on *Tulipa gesneriana* utilized gamma doses at 0, 5, 10, 20, 40, 60, 80, and 100 Gy. They observed four different flower color variants, and Radwan (2023) mutated *gypsophila elegans* seeds with sex doses of gamma radiation at 10–60 Gy. He recorded several changes in flowers, such as a flower with four petals, slender petals, a colored pink flower, a deformed flower, a colored pink flower with biforked petals over two generations.

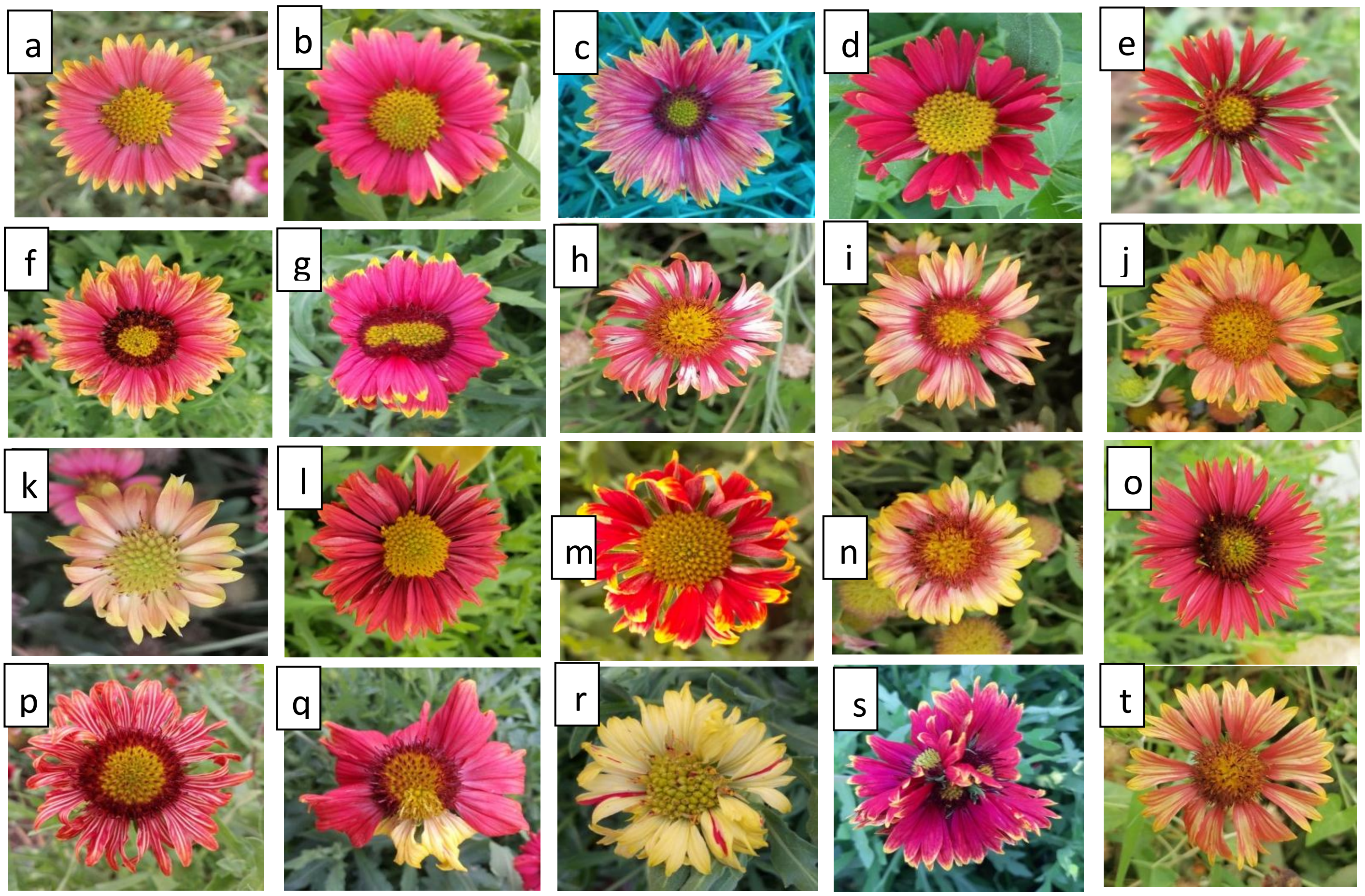



**Fig. 3. Showing the plant abnormalities of *G. pulchella* as affected by different doses of gamma rays on inflorescence color in $M_3$ generation.**

(**a, 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 others red color. **(c**, **20 Gy** variegated radial flowers red with yellow. **(d, 30** Gy dark maroon radial flowers coiled from the bottom. **(e, 40** Gy slender ray flowers**. (f, 40** Gy semi-double inflorescence, radial red flowers with orange interlacing in the upper part of the petals. **(g, 40** Gy fasciation inflorescence**. (h, 40** Gy variegated ray flowers**. (i, 40** Gy multicolored radial flowers intertwining red with orange and yellow**. (j, 40** Gy orange radial flowers tinged with light red**. (k, 40** Gy radial yellow flowers tinged with light pink. (**l, 50** Gy Semi-double inflorescence. (**m, 50** Gy bright red radial flowers with yellow tips, funnel-shaped coiled petals. (**n, 50** Gy yellow radial flowers tinged with red at the bottom. (**o, 60** Gy red radial flowers with acute tips. **(p, 60** Gy radial flowers variegated with longitudinal stripes in white with red colors. (**q, 60** Gy chimeric mutation, four petals are yellow and the others are red. (**r, 60** Gy chimeric mutation, some of the petals are stained red and others are pure yellow. (**s, 60** Gy three inflorescences on the same stalk. (**t, 60** Gy light red and orange radial flowers intertwined together with slender petals**.**

**g. Anatomical structure of the leaves and inflorescences:**

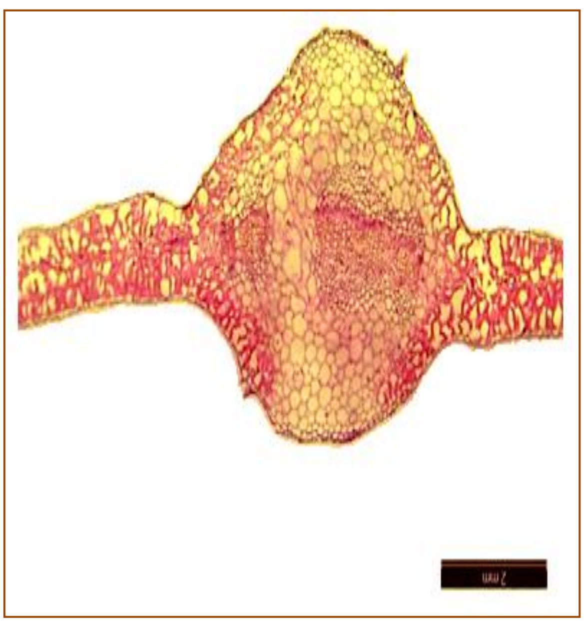
**1- Effect of gamma on the anatomical structure of the leaves:**

According to the data in Table (5) and Fig. (4) mentioned that, the dose of gamma radiation at 40 Gy gave the greatest number of vascular bundles with value of 4.00 vascular bundles, compared to the control plants that were given (2.00 vascular bundles), and other gamma radiation doses that were given for each treatment (3.00 vascular bundles). Also, data indicated that using a gamma dose of 40 Gy produced the greatest value of lamina thickness (1.25 mm) in comparison with the control (1.20 mm). On the other hand, the other doses of gamma at 20 Gy followed by 10 Gy and 60 Gy decreased the values of thickness of lamina to 0.95, 0.90 and 0.85 mm, respectively. Concerning the effect of gamma rays on the thickness of the midvein, the results indicated that utilizing the doses of 10 Gy followed by 40 Gy as well as 20 Gy produced the largest values of the midvein thickness (5.60, 5.30 and 5.20 mm, respectively) compared to (4.50 mm) for the control plants. Whereas, the lowest value (4.00 mm) was produced with the dose of 60 Gy.

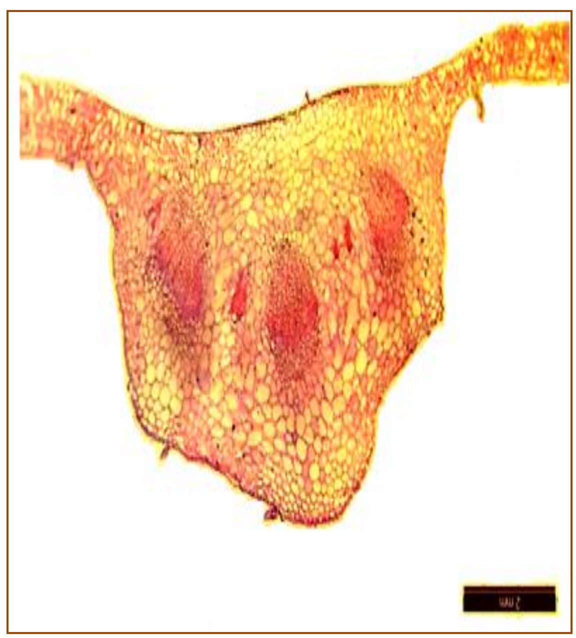
**Table (5) Effect of gamma radiation treatments on No. of vascular bundle, thickness of lamina and thickness of midvein of *G. pulchella* plant, during the $M_3$ generation (2021/2022).**

| Treatments | No. of vascular | Thickness of lamina (blade) | Thickness of midvein |
|---|---|---|---|
| **Control** | 2.00 | 1.20 | 4.50 |
| **10 Gy** | 3.00 | 0.90 | 5.60 |
| **20 Gy** | 3.00 | 0.95 | 5.20 |
| **40 Gy** | 4.00 | 1.25 | 5.30 |
| **60 Gy** | 3.00 | 0.85 | 4.00 |

**Gy=Gray** **mm=Millimeter**

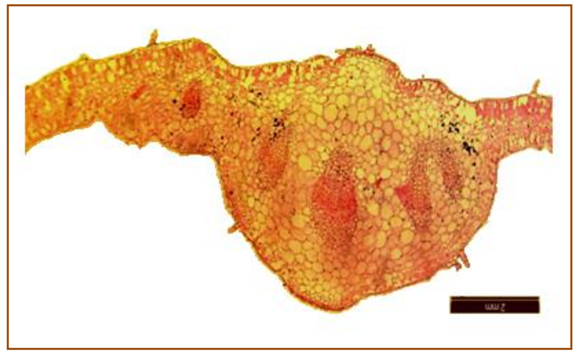


**Control**



**10 Gy**

**20 Gy**



**40 Gy**

**60 Gy**

**Fig. 4. Anatomical structure of leaves (mm) in *G. pulchella* as affected by gamma radiation treatments compared to the control, (Transverse section through the leaves of the third developed leaf from the third branch of the upper part of the main stem of the plant (Magnification 20x)).**

In this regard, it was suggested that the plant cells and tissues have a variety of functions ranging from storage and support to photosynthesis. Apart from the xylem and phloem present in its vascular bundles, the leaves mainly consist of parenchyma cells, so the large size of the cells and the increase in their number as a result of gamma irradiation play an important role in the process of photosynthesis and the storage of food resulting from this process and thus improve plant growth. Increasing the vascular bundle with their contents number of vessels and rows of xylem is responsible for transporting water and salts needed by the plant from the roots, then it goes to the stem and then spreads to the leaves, and thus has an important role in feeding the plant and providing it with strength and support, which leads to improved plant growth and increased yield. Also, Dickison (2000) stated that the anatomical structure of the leaf can be changed as a result to gamma radiation that is ionising in nature. Although the extent of the growth varied and did not follow a pattern of increasing doses of irradiation, gamma ray irradiation is known to increase the thickness of lamina, epidermis, palisade and leaves in certain individuals, and Harahap (2005) on *Garcinia mangostana* plant, suggested that the mutant's anatomical structure can be used to explain variations in how some processes are controlled genetically; physiological or genetic alterations are anticipated in cells that can grow following exposure to radiation.

**2- Effect of gamma radiation on the anatomical structure of the inflorescences:**

The obtained data in Table (6) and Fig. (5) indicated that treating the plants with gamma radiation at 10 and 20 Gy formed the largest number of disc flower rows, which were given for each dose (13 rows) compared to the control plants (11 rows). As for the flowering bud diameter (FBD), it was found that the doses of 10 as well as 20 Gy produced the biggest flowering buds, giving values of 9.00 and 8.00 mm respectively, compared to the control (7.80 mm). Also, it was found that the doses of 10 and 20 Gy gave the largest values of the receptacle diameter (4.80 and 4.20 mm, respectively), compared to the untreated plants (3.90 mm). In this regard, Wi *et al.* (2007) reported that low doses of gamma radiation had a stimulating impact, which could be attributable to changes in hormones in plant cells or an increase in the cells' antioxidative capacity. Chakravarty and Sen (2001) on *Scilla indica* plant, stated that low doses of gamma rays enhanced the growth rate may be attributed to stimulate of cells division or cell elongation. Khangyldin (1967) suggested that low doses of gamma radiation increased the kinetin to auxin ratio in buds, leaves, flowers and shoots; gamma irradiation or kinetin can also create the same hormonal equilibrium, the growth hormone kinetin was boosted, resulting in an increasing the number of branches and leaves, and Pitirmovae (1979) reported that gamma radiation caused changes in metabolic processes that affect phytohormone growth or nucleic acid synthesis. In this regard, Widiastuti *et al.* (2010) on *Garcinia mangostana*, found that the size of the leaves changed after exposure to gamma rays, which was due to alterations in the number and/or size of the cells; because a thicker lamina (blade) can reflect sunlight and slow water transpiration, thicker lamina is more likely to contain traits that make plants more tolerant to drought. It has been suggested that low and medium levels of gamma doses increased the cell size and number as well as increased vessel number, which can be utilized as an indirect selection criterion for effectiveness, because the cuticle also acts to protect plants from diseases and pests. In this respect, Bajpay and Dwivedi (2019) on *Dendranthema grandiflora*, applied gamma radiation at 0, 100, 150, 200, 250, 300, 350, 400 and 450 Gy, and they found that the small level of gamma resulted in the highest percentage of leaf alteration and stomata abnormalities, on the other hand, the large levels of gamma radiation caused increases in anatomical feature abnormalities.

**Table 6. Effect of gamma radiation treatments on No. of disc flowers rows, flowering bud diameter and receptacle diameter of *G. pulchella* plant, during the $M_3$ generation (2021/2022).**

| Treatments | No. of disc flowers | Flowering bud diameter (FBD) | Receptacle diameter |
|---|---|---|---|
| **Control** | 11.00 | 7.80 | 3.90 |
| **10 Gy** | 13.00 | 9.00 | 4.80 |
| **20 Gy** | 13.00 | 8.00 | 4.20 |

**Gy=Gray mm=Millimeter $M_3$= Third generation**


**Control** **10 Gy** **20 Gy**

**Fig. 5. Anatomical structure of flowers (mm) in *G. pulchella* as affected by gamma radiation treatments compared to the control, (Longitudinal section through the flowering bud (Magnification 20x)).**

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