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Evaluation of mutation breeding by gamma rays on M_3 generation plants for improvement of *Gypsophila elegans* M.Bieb plant

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ABSTRACT

Gypsophila is one of the basic plants of ornamentals. It is valued as a cut flower in floristry to add as a filler to flower bouquets and in gardens landscaping. This study focused on evaluation of mutation breeding (MB) by gamma rays on M_3 plants for improvement of *G. elegans* plant and determined of proper doses for (MB) which can increase the turnout of its market. Irradiation treatments were performed on the seeds in M_1 only, the seeds were mutated with six different dosages from (10 – 60 Gy) using Gamma-1 type cobalt60. The Results declared that that control plants gave the maximum germination percentage; in contrast, the lowest value was achieved with 60 Gy. Using gamma at 10, 20 and 40 Gy, improved the morphological characters, such as plant height, leaves number, stems diameter, roots length, early flowering, and flowers production. Meanwhile, 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. The correlation relationship among the plant height and all morphological characters, was medium significant correlation (r = 50 to 70) to very high (r=70 to 90) except for No. of days to flowering and No. of internodes which showed a high significant negative correlation with them. Many mutants in flower color, and shape were obtained.

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Using gamma radiation increased (RWC) above 70%, but decreased (EL) and (MSI). Utilizing gamma at 10 and 20 Gy gave the highest values of anatomical characters.

Key words: *Gypsophila elegans*, gamma rays, improvement, morphological characters, anatomical structure, (RWC), (EL) and (MSI)

INTRODUCTION

Gypsophila elegans is an ornamental plant, native to Asia and Europe; it is member of Caryophyllaceae family. Plants of the genus are comprises about 150 species of annual or perennial herbaceous plants, known as baby's breath. Genus of gypsophila is commercially cultivated for several uses, including, floristry, cut flower arrangements to add as a filler to flower bouquets, gardens landscaping, and herbal medicine (Korkmaz and Özçelik, 2011). It contains biological compounds such as triterpene, saponins, flavonoids, sterols are important for the pharmaceutical industries (Zdraveva *et al*., 2015). Flowers are single (five petals) on branching stems (white or light pink). Leaves are narrow, grey-green, opposite, lance shaped, smooth. Stems are highly branched and swollen at the nodes. The fruit is a rounded or oval capsule, contains brown or black seeds (Korkmaz *et al*., 2012).

Classical breeding and selection studies have caused a serious contraction in the genetic diversity of plant species (Tanksley and McCouch, 1997). In recent years, breeding trials aimed at obtaining uniform plants accelerated this process and caused many crops to become more sensitive to diseases, pesticides and abiotic stress conditions (Plucknett *et al.*, 1983). The genetic bottle-neck that occurs as a result of the continuous use of existing populations leads plant breeders to modernized breeding technologies. Mutation breeding has been an alternative technique preferred by breeders as it allows the possibility to form characteristics that do not exist in the nature or lost throughout the evolution. It is possible to create new variations in a short time with the use of the mutation breeding method. The most used mutation breeding agent is physical mutagens such as ultraviolet (UV) light which leads to breaks on DNA double strain and deletions, gamma rays and neutrons have high energy radiation applications (Koornneef, 2002). Physical mutagens are an alternative breeding method to classical breeding methods as well as genetically modified organisms because their applications are relatively safe and cost-effective (Jain, 2010). According to International Atomic Energy Agency (IAEA, 2015) data, there are at least 3233 mutant cultivars by gamma radiation. The ionizing radiation method, which began to be used in the early 20th century, plays an important role in the development of superior plants in more than 50 years of plant breeding studies (Kharkwal, 2012).

Although there are several techniques used for mutation breeding, gamma ray technique is one of the most used 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 (Sağel *et al*., 2003, and Sağel *et al*., 2009). Gamma rays are widely used mutagen for their simple application, having short wavelength with high penetrable power, high mutation frequency and less risky to health than chemical mutagens because they require no application to remove mutagen from the material (Khan *et al*., 2000), because they are non-toxic and do not require detoxification after implementation (Mba, 2013). The ease of application plays an important role on widely spreading of the technique. 90% of the obtained mutant cultivars were obtained with this method (64% with gamma-rays, 22% with X-rays) (Jain, 2005, and Jain, 2010). Many new cultivars have developed in *Anthurium* (Puchooa, 2005) and *Coriander* (Salve and More, 2014), also, many novel characters have obtained in carnation (Sabaghi *et al*., 2018) *Tagetes* (Sarhan *et al*., 2019), *Chrysanthemum* (Chen *et al*., 2020), *Calendula* (Elmenbawy *et al*., 2020), *Helichrysum* (Eid *et al*., 2021), orchid (Hartati *et al*., 2021), *Tulipa gesneriana* (Li., 2022), *Catharanthus* (Al-Haidari and Al-Tamimi, 2023), *Borago* (Fayed, 2022), and *Gaillardia* (El-Khateeb *et al*., 2023). The objective of this study is to improve the morphological characters and determine the proper dose of gamma irradiation for the mutation breeding program of *G. elegans* plant in M_3.

MATERIALS AND METHODS

Study materials: The seeds of *G. elegans* (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 of 2021/22 (M_3).

Treatment of seeds: Irradiation treatments were performed on the seeds in the first generation (M_1) only. The seeds of *G. elegans* (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

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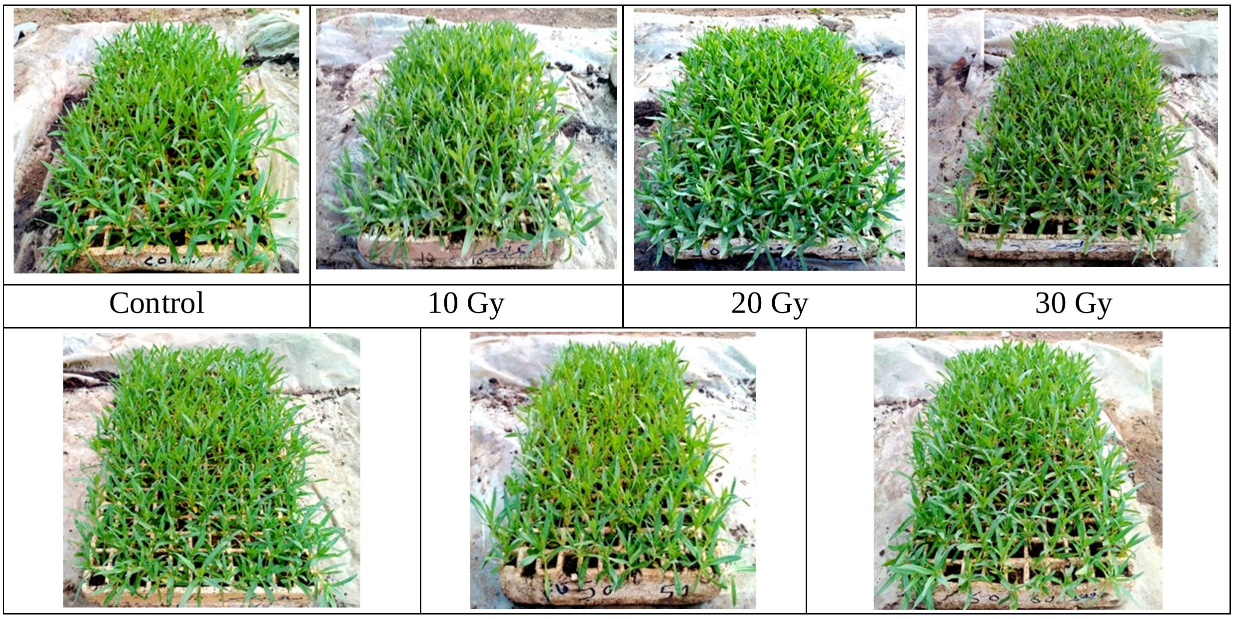
Commission-united irradiation-Gamma, Nasr city, Egypt, at the doses of (0.0, 10, 20, 30, 40, 50 and 60 Gy). The control seeds were kept without irradiation. Gray gamma-irradiation doses were done using Indian gamma cell (Ge 4000 A), type cobalt 60 (Co60) and dose rate at 1.107 KGy/h.

Preparing seedlings, transplantation and growth conditions for the selected mutants of M_3 (2021/22)

The mass selection of seeds in 2019/20 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 2020/21 (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 treated plants in the M_2 to obtain the third mutative generation seeds 2021/22 (M_3), 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. 1) on 5, October 2021) to produce seedling. After 8 days of sowing seeds began germination, and 45 days later of sowing, uniform seedlings (12-14 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), as every plot (3.5 x 1.8 m) contained 21 hills /plot.



Control | 10 Gy | 20 Gy | 30 Gy

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40 Gy	50 Gy	60 Gy

Fig. 1. The seedlings stage of *G. elegans* in plastic trays as affected by different doses of gamma rays in M_3.

Agricultural practices: All the recommended cultural practices namely, irrigation and fertilizer, 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) (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. elegans* plants which grown until the flower opening reached 50%, that is, about a month after the flowers start to appear for each treatment:

(a) Seed germination (%), the germination percentage of seeds was measured using the following equation; Germination (%) = $\frac{\text{No.of seeds germinated}}{\text{Total No.of seeds sown for germination}} \times 100$

(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), number, diameter and stalk length of flowers/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)** Flowers abnormalities.

(g) Anatomical structure of the leaves and flowers: 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 (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, (Nassar and El-Sahar, 1998). The slides were microscopically examined; counts and measurements (µm) of the different tissues (leaves and flowers) were taken and

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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 analysed 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

a. Seed germination (%)

The results in (Table 1) indicated that all doses of gamma rays reduced the germination % when compared to the control, and it was observed that the low doses of gamma enhanced the seeds germination as compared to the high doses, and the maximum germination % (94.11 % and 93.33 %) was achieved with the doses of 20 followed by10 Gy, respectively. But, the high doses of 50 and 60 Gy gave the minimum germination % (88.21 % and 85.15%), respectively, compared to (97.03%) for the control.

b. Vegetative characters

It could be mentioned from the data in (Table 1) that treating plants with the doses of 10, 20, 30 and 40 Gy produced the tallest plants (125.65, 123.70, 115.60 and 118.33 cm), respectively, over the control which recorded (113.30 cm). Meanwhile, the shortest plants (106.25 and 95.48 cm) were found with the doses of 50 and 60 Gy, respectively. Also, data indicated that, the doses of 10 as well as 20 then 40 Gy increased the ramification of the plants and leaves formation, to the highest values, giving (14.30, 13.85 and 12.50 branches) and (620.36, 590.48 and 575.31 leaves), with increasing of (32.77%, 28.59% and 16.06% for branches) and (15.78%, 10.20% and 7.37% for leaves), respectively, compared to (10.77 branches and 535.80 leaves) for the control.

On the other hand, with increasing gamma doses to 50 and 60 Gy produced the lowest ones, giving (7.63 and 8.80 branches) and (406.60 and 423.36 leaves), respectively. The widest diameter of stems (1.78 cm) was achieved with the lowest dose of 10 Gy with an increase %

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(31.85%). On contrast, with increasing the radiation dose to 60 Gy, the smallest diameter (1.21 cm) was produced with a decrease % (10.37%), compared to (1.35 cm) for the control. Considering the results of internodes number in 30 cm, the low doses of 10 and 20 Gy formed long internodes, thus its number decreased (7.81 and 9.01), with decrement % (30.57% and 19.91%), respectively, compared to the control that recorded (11.25). On the contrary, short internodes was formed with the high doses of 60 followed by 50 Gy, thus its number increased to (14.25 and 13.36) with increment % (18.75% and 26.66%), respectively, compared to the control. According data of average root length (cm) presented in (Table 2), revealed that the longest roots (16.40 and 14.33 cm) were produced with the low doses of radiation at 10 and 20 Gy by increasing % (36.32% and 19.11%), respectively. While, the other doses of gamma did not make any significant differences among them and the control.

Table 1. Effect of gamma rays treatments on seed germination, plant height, No. of main branches/plant, No. of leaves/plant, stem diameter and No. of internodes of *G. elegans* plant, during the M_3 generation (2021/22).

Treatments	Seed germination (%)	Plant height (cm)	No. of main branches/ plant	No. of leaves/ plant	Stem diameter (cm)	No. of internodes in 30 cm
Control	97.03 a	113.30 d	10.77 d	535.80 c	1.35 bc	11.25 b
10 Gy	93.33 b	125.65 a	14.30 a	620.36 a	1.78 a	7.81 d
20 Gy	94.11 b	123.70 a	13.85 ab	590.48 ab	1.60 ab	9.01 cd
30 Gy	89.16 d	115.60 c	11.48 cd	560.03 bc	1.33 c	11.30 b
40 Gy	91.25 c	118.33 b	12.50 bc	575.31 b	1.45 bc	10.25 bc
50 Gy	88.21 d	106.25 e	7.63 e	406.60 d	1.30 c	13.36 a
60 Gy	85.15 e	95.48 f	8.80 e	423.36 d	1.21 c	14.25 a

Table 2. Effect of gamma rays treatments on average root length, No. of days to flowering, number and diameter of flowers/plant, and flowers stalk length of *G. elegans* plant, during the M_3 generation (2021/22).

Treatments	Average root length (cm)	No. of days to flowering (DPF)	No. of flowers /plant	Flowers diameter (cm)	Flowers stalk length (cm)
Control	12.03 cd	114.33 c	553.75 d	1.77 c	24.58 c
10 Gy	16.40 a	98.10 e	660.03 a	2.15 a	30.05 a

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20 Gy	14.33 b	100.50 e	625.49 b	2.03 ab	27.78 b
30 Gy	12.40 bcd	115.30 c	562.60 d	1.77 c	21.25 d
40 Gy	13.66 bc	111.00 d	596.16 c	1.96 b	26.11 bc
50 Gy	11.30 d	124.55 b	454.08 f	1.67 c	18.41 e
60 Gy	12.29 bcd	132.25 a	470.56 e	1.70 c	18.93e

c. Flowering characters

Data recorded in (Table 2) showed that, using gamma at 10, 20 and 40 Gy produced the first flowers, and shortened the vegetative growth phase to (98.10, 100.50 and 111.00 day), respectively, compared to (114.33 day) for the control. Whilst, the doses of 50 and 60 Gy increased the number of days elapsed to flowering, by (124.55 and 132.25 day), respectively. Also, (Table 2) showed that, the low doses of 10 and 20 Gy significantly increased the flowers production /plant, giving the maximum number (660.03 and 625.49 flowers), the largest flowers (2.15 and 2.03 cm) and the longest flowers stalks (30.05 and 27.78 cm) with increment of (19.19% and 12.95% for flowers number) and (21.46% and 14.68% for flowers diameter) and (22.25% and13.01% for flowers stalk length), respectively, over to the control. On the other hand, the high doses of 50 and 60 Gy produced the minimum ones.

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

Regarding data in (Table 3) detected that the correlation was medium significant among the seed germination (%) and following characters, plant height, number of branches, number of leaves and number of flowers, and low correlation with stem diameter. While, the correlation was low and not significant with average root length. The correlation relationship between the plant height and all vegetative and flowering characters, was medium significant correlation (r = 50 to 70) to high significant correlation (r=70 to 90). The correlation coefficients were very high significant (r ≥ 90) between plant height and number of flowers/plant. Also, data in (Table 3) showed that, the number of internodes was negatively correlated with all the vegetative and floral studied characters, except for the number of days to flowering, which showed a high significant positive correlation with it. The correlation was a medium (r = 50 to 70) to a high (r =

70 to 90) relationship among all flowering characters correlated with each other. The number of days from planting to flowering (DPF) was negatively correlated with all the vegetative and floral characters.

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	SG (%)	PH (cm)	NB/p	NL/p	SD (cm)	ARL (cm)	NI	DPF	NF/p	FSL (cm)	FD (cm)
SG (%)											
PH (cm)	67.57**										
NB/p	55.21**	89.21**									
NL/p	65.63**	89.17**	91.27**								
SD (cm)	45.93*	76.50**	79.50**	68.87**							
ARL (cm)	36.37^{ns}	67.02**	78.18**	71.88**	74.45**						
NI	-64.60**	-87.76**	-83.36**	-86.28**	-83.35**	-70.21**					
DPF	-71.30**	-90.55**	-82.68**	-89.53**	-81.21**	-70.04**	-92.59**				
NF/p	64.83**	91.33**	93.55**	95.79**	78.50**	78.26**	-91.24**	-93.24**			

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

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FSL (cm)	72.77**	82.39**	83.93**	87.72**	76.97**	73.26**	-91.56**	-92.15**	92.10**	
FD (cm)	47.02*	75.59**	80.22**	80.51**	77.64**	79.84**	-82.52**	-85.56**	85.69**	87.01**

***Significant at P < 0.05, ****Significant at **P < 0.01, ns=** Nun significant**, 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)**, NI =** No. of internodes**, DPF =**No. of days to flowering, **NF/p =**No. of flowers/ plant**, FSL (cm)=**Flower stalk length (cm) and **FD (cm)=**Flower diameter (cm)**.**

e. Physical characters (Water status in leaves)

1. Relative water content (RWC)

According to the results of relative water content (RWC) in (Table 4) indicated that, all doses of gamma radiation induced significant increases in (RWC) compared to the control. The highest percentages of RWC (74.72%, 75.49%, 75.77% and 74.50%) were recorded with the doses of 10, 20, 30 and 40 Gy, respectively, compared to (68.04%) for the control. Also, regarding the results of this study, mentioned that all gamma doses of radiation were able to keep RWC above 60%.

2. Electrolyte leakage (EL)

Gamma radiations at the doses of 10, 20, 30 and 50 Gy significantly decreased the electrolyte leakage (EL), giving (29.04%, 31.24%, 29.81% and 30.75%), respectively, compared to (32.71%) for the control. On the other hand, gamma radiation at the doses of 40 and 60 Gy, did not make significant differences compared to the control, as showed in (Table 4), also, data indicated that regardless of the control, all doses of gamma resulted in a reduction in (EL) except for the doses in 40 and 60 Gy, which gave the percentages of (31.70% and 33.73%).

3. Membrane stability index (MSI)

According to the results in (Table 4), revealed that all doses of gamma radiation decreased the (MSI %), compared to for the untreated plants**,** and formed cells with more stable membranes than those that were exposed to the radiation. Regardless of the control treatment, by comparing the radiation doses with each other, it was observed that, the highest percentage (70.95%) was recorded with the smallest dose of 10 Gy. Conversely, the lowest percentage (64.26%) was recorded with the largest dose of 60 Gy.

Table 4. Effect of gamma rays treatments on relative water content (%), electrolyte leakage (%) and membrane stability index (%) of *G. elegans* plant, during the M_3 generation (2021/22).

Treatments	Relative water content RWC (%)	Electrolyte leakage EL (%)	Membrane stability index MSI (%)
Control	68.04 d	32.71 ab	82.33 a
10 Gy	74.72 a	29.04 e	70.95 b
20 Gy	75.49 a	31.24 c	67.75 c
30 Gy	75.77 a	29.81 de	70.32 bc
40 Gy	74.50 a	31.70 bc	68.18 bc
50 Gy	72.73 b	30.75 cd	70.24 bc
60 Gy	71.26 c	33.73 a	64.26 d

f. The flowers abnormalities during the M_3

The flowers abnormalities pictured in (Fig. 2) illustrated that using gamma treatments caused many variants in color and shape of the flowers (whether in shape of the petals or their coloration in pink such as flower with four petals, slender petals, colored pink flower, deformed

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flower and colored pink flower with biforked petal, compared with non-irradiated plants, Besides, the high gamma doses of 40 and 60 Gy recorded the highest number of mutants in the flowers. 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.

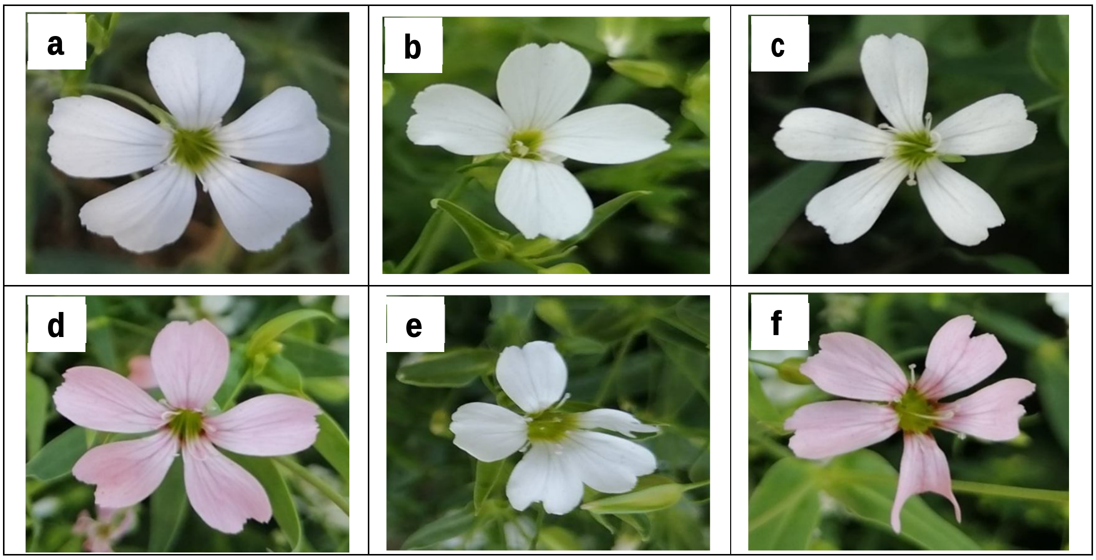

Fig. 2. The flowers abnormalities of *G. elegans* as affected by different doses of gamma rays on inflorescence in M_3 generation.
(a) Control original flowers, (Serrulate apex). **(b)** 10 Gy, (Flower with four petals). **(c)** 30 Gy, (Slender petals). **(d)** 40 Gy, (Colored pink flower). **(e)** 60 Gy, (Deformed flower). **(f)** 60 Gy, (Colored pink flower with biforked petal).

g. Anatomical structure of the leaves and flowers

1. Effect of gamma on anatomical structure of the leaves

a. No. of xylem rows

The results presented in (Table 5 and Fig. 3) showed that using gamma radiation at the dose of 10 Gy enhanced the number of xylem rows of leaf, and gave the highest value by (28 rows), as compared to the other gamma radiation doses and the control gave (26 rows). On the contrary, the least value was (17 rows) produced when treating plants with the dose of 60 Gy.

b. Thickness of lamina (blade) (µm)

It could be observed from data presented in (Table 5 and Fig. 3) that irradiating plants with the doses of gamma radiation at 10 Gy followed by 40 then 20 Gy increased the thickness of lamina, giving values of (437.50, 437.50 and 425.00 µm), respectively, compared to the control (390.00 µm). On the other hand, irradiating plants with the doses of 30 Gy and 60 Gy produced the lowest thickness of lamina, giving values of (377.60 and 312.50 µm), respectively, compared to the control (390.00 µm).

Table 5. Effect of gamma radiation treatments on No. of xylem rows, thickness of lamina, thickness of midvein, length of vascular bundle and width of vascular bundle of *G. elegans* plant, during the M_3 generation (2021/22).

Treatments	No. of xylem rows	Thickness of lamina (blade) (µm)	Thickness of midvein (µm)	Dimension of bundle	
				Length of vascular bundle (µm)	Width of vascular bundle (µm)
Control	26	390.00	1250	1000	275
10 Gy	28	437.50	1375	950	325
20 Gy	25	425.00	1725	1250	300
30 Gy	22	377.60	1025	825	250
40 Gy	24	437.50	1500	925	350
60 Gy	17	312.50	875	625	150

Gy=Gray µm=Micrometer M_3= Third generation

c. Thickness of midvein (µm)

Utilizing gamma at 20 Gy followed by 40 as well as 10 Gy resulted in the greatest values of midvein thickness (1725, 1500 and 1375 µm), respectively, compared to (1250 µm) for the control. On contrast, the lowest values of (875 and 1025 µm) were produced with the doses of 60 Gy followed by 30 Gy, respectively, compared to the control, (Table 5 and Fig. 3).

d. Dimension of vascular bundle (µm)

The dose of 20 Gy produced the maximum length of vascular bundle by (1250 µm). Whilst, the shortest of vascular bundle of (625 µm) was found with 60 Gy, compared to (1000 µm) for the control. Using gamma at 40 Gy followed 10 Gy then 20 Gy increased the width of bundle by (350, 325 and 300µm) compared to the control (275 µm). On contrast, the dose of 60 Gy produced the smallest width of bundle by (150 µm), as showed in (Table 5 and Fig. 3).

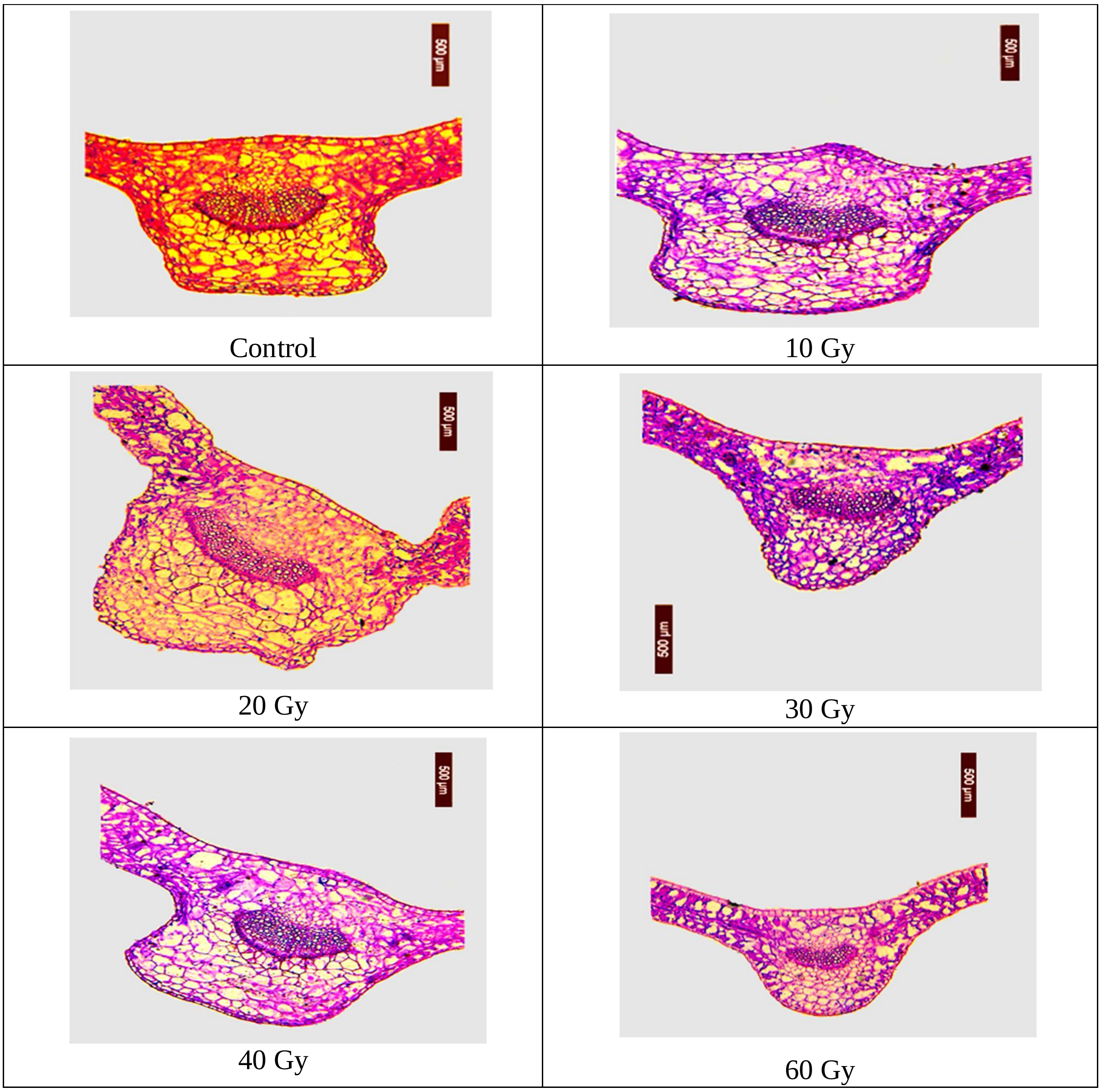

Fig. 3. Anatomical structure of leaves (µm) in *G. elegans* plant as affected by gamma radiation doses compared the control, (Transverse section through the leaves of the third developed leaf from the third branch of the upper part of the main stem of the plant (Magnification 80x)).

2. Effect of gamma radiation on anatomical structure of the flowers

It could be observed from data presented in (Table 6 and Fig. 4) that, the low doses of 20 followed by 10 Gy had a positive effect, and resulted in the largest values of flowering bud

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diameter (FBD) by (9.75 and 8.00 mm), respectively, compared to the control (6.00 mm). Also, the doses of 20 as well as 10 Gy of gamma rays gave the largest receptacle diameter by (2.30 and 2.20 mm), respectively, compared to the control (1.75 mm).

Table 6. Effect of gamma radiation treatments on flowering bud diameter (FBD) and receptacle diameter of *G. elegans* plant, during the M_3 generation (2021/22).

Treatments	Flowering bud diameter (FBD) (mm)	Receptacle diameter (mm)
Control	6.00	1.75
10 Gy	8.00	2.20
20 Gy	9.75	2.30

Gy=Gray mm=Millimeter M_3= Third generation

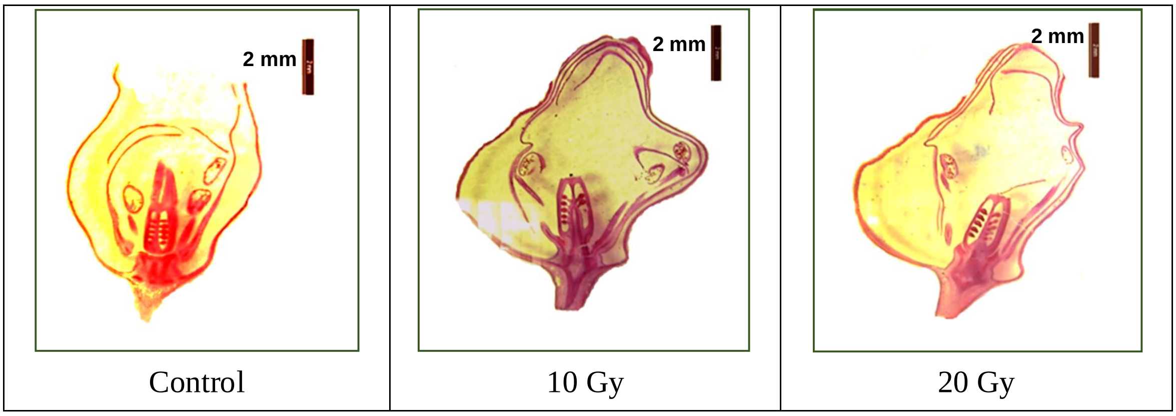

Fig. 4. Anatomical structure of flowers (mm) in *G. pulchella* as affected by gamma radiation treatments compared to the control, (Longitudinal section through the flowering bud (Magnification 20x)).

DISCUSSION

Gamma rays as ionizing radiation are widely used for improving the vegetative growth and flowering of ornamental plants and to induced genetically diversity or improve many useful traits of the plant. This technique does not produce a new genetic combination from parental genes, but it creates new gene combinations with high frequency of mutation. Some of these mutations might be useful when they have higher economical values or create a new trait (Abdullah *et al.,* 2010). Nowadays, the no. of new cultivars derived by induction increase constantly with new traits as plant size, chape, color and size of flowers and time of flowering, and resistance to pathogens. (Maluszynski *et al*., 1995).

From our results on the effects of gamma irradiation on gypsophila plant, it can be indicated that using gamma rays reduced germination percentage compared to the control. Low doses of gamma rays 10 as well as 20 Gy enhanced germination percentage, but 50 and 60 Gy decreased it to the lowest values. The results are in agreement with those reported by (Radwan, 2017) on *Helichrysum bracteatum*, utilized eight different doses of gamma (5–40 Gy), all doses reduced seeds germination compared to the control; (Chedeo and Wamaedeesa 2021) on garden balsam plant, they noticed that gamma doses decreased germination percentage, and (El-Khateeb *et al*., 2023) mutated *Gaillardia pulchella* seeds with six doses from (10 – 60 Gy), and they recorded a reduction in seed germination compared to the control. 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, as suggested by (Melki and Marouani, 2010). 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). Also, improvement in germination and growth traits of plants exposed to low doses of gamma irradiation may be attributed to positive mutational effects on genes controlling these traits, rapid DNA repair mechanism and stimulation of hormones and enzymes which are actively involved in germination and growth processes. Lower doses might possibly accelerate cell division in meristematic tissues which could contribute to improved germination and plant growth (Dhakshanamoorthy *et al*., 2010).

Concerning the effects of gamma irradiation on vegetative growth and flowering character, it can be revealed that most of doses of gamma rays (10 -20 Gy) gave the highest values of plant height, stems diameter, ramification of plant, leaves formation, roots length and induced early flowering, but the highest level of 50 and 60 Gy had a negative effect on these characters and reduced them to the lowest values and prolonged the vegetative growth phase. In this regard, the stimulation effects of low doses of gamma rays on vegetative growth traits of many plant species have been reported by many studies, (Omar, 2016) on *Lathyrus odoratus*, found that 10 Gy of gamma enhanced the plant height and branches number; (Radwan, 2017) on *Helichrysum bracteatum*, he concluded that dose of 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) on *Jasminum grandiflorum*, they observed that the low doses of 10, 15, 20 and 25 Gy, enhanced vegetative growth characters of the plant; (Rifnas *et al*., 2020) on *Allamanda cathartica*, they discovered that the low doses of 0, 17, 18, 19, 20 and 21 Gy increased plant height; (Hartati *et al*., 2021) they recorded a significant increases in the morphological characteristics of the plant compared to the control when mutated the seeds of *Vanda orchid* with gamma doses of 10 and 20 Gy. (Hajizadeh *et al*., 2022) on lilium bulbs, they reported that low deses induced a positive effect on the vegetative growth characteristics of the plant, and (Radwan, 2023) who found that the low doses of 10 and 20 Gy increased the most vegetative growth characters of *Gaillardia pulchella*, while the doses of 50 and 60 Gy decreased them.

In this respect, low doses of gamma radiation had a stimulating effect, which could be attributed to changes in hormones in plant cells or an increase in the cells' antioxidative capacity and enhanced growth rate by speeding cell division (Wi *et al*., 2007). Moreover, (Jan *et al*., 2013) reported that, gamma irradiation at low doses exhibit a positive effect on many traits of plant growth, in contrast the high dose owing to causes a marked direct or indirect damages in plants plant morphology and physiology, 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. (Marcu *et al*., 2013a) argued that creation of free radicals in cells in response to gamma irradiation can affect proteins, lipids, enzymes, cellular molecules and ionization of water present in cells which may cause increased antioxidant responses and alteration cell membrane permeability potentials. These changes can trigger growth abnormalities. Also, (Kumari and Kumar, 2015) on gladiolus plant, stated 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.

It was concluded from the results that using gamma showed a great effect on flowering characters. The control plants began flowering after 114.33 days after sowing the seeds, whereas, the high levels of gamma (50 and 60 Gy) were the most effective in decreasing the No. of flowers/plant, it diameters, gave the shortest flowers stalks and prolonged the vegetative growth phase (VGP), meanwhile, the low doses of 10 and 20 Gy markedly shortened the VGP and enhanced flowering stage and increased flowers production to the highest values. The results obtained are in consistent with (Abdel Mageed *et al*., 2016) mentioned that the low dose of 10 Gy enhanced the flowers formation in *Lathyrus odoratus*; (Bhusari *et al*., 2017) on *Tagetes erecta*, who reported that low doses of gamma improved flowering parameters; (Sabaghi *et al*. 2018) irradiated carnation 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 flowers number and it diameter, and (Radwan, 2023) on *Gaillardia pulchella*, who noticed that the number, diameter and stalk length of flowers increased and early flowering with the doses of 10 and 20 Gy, while the doses of 50 and 60 Gy reduced them to the minimum values and prolonged vegetative growth phase.

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; (Ismael and Mahmoud, 2015) concluded 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; (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 increased hormone synthesis and therefore on bud production, and (Shukla *et al*., 2018) on *Gladiolus grandiflorus*, they 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.

As for the relationship between the plant height and all vegetative and flowering characters, was medium positive significant correlation (r = 50 to 70) to high (r=70 to 90) except for the number of days to flowering and No. of internodes which showed a high significant negative correlation with it. In this regard, (Carpici and Celik, 2010) suggested that the correlation coefficient in statistics is one of the common statistical methods that are used to determine how much and how the relationship is between two or more variables. Also, define correlation as the correlative process of change; this is because when one of the two variables changes, the other variable follows, as reported by (Silva *et al*., 2016).

The results of physical characters indicated that, all doses of gamma radiation induced significant increases in (RWC) above 70% over to the control. 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 the high doses, and (Akhtar, 2015) mutated *Solanum lycopersicum* seeds 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 stated that, treating plants 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, the plants were exposed to low doses of gamma may be produced a number of heat stable proteins linked to stress tolerance. Also, (Nikièma *et al*., 2020) suggested that the low gamma doses result in increases in (RWC). Furthermore, 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 gamma rays at the doses of 200, 300 and 400 Gy, they were found to be more resistant to radiation.

The doses of 10, 20, 30 and 50 Gy significantly decreased (EL) compared to the control, this reduction in (EL) may be due to cell membrane obstruction produced by aggregation and

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denaturation of intracellular biomacromolecules. These results are in good harmony with (Hajizadeh, *et al*., 2022) on lily cut flowers, irradiated the bulbs with gamma doses of control, 10, 20, 30, 40 and 50 Gy, and they revealed that the (EL%) significantly decreased after irradiation treatments. 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, and (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. As for (MSI%), all doses of gamma decreased it, compared to control plants, and formed cells with more stable membranes than those that were exposed to the radiation, it could be suggested that the reduction in the stability of the cell membrane after exposed to radiation may be attributed to, the membrane has been damaged and electrolytes have leaked from it. It appears to be due to ion reflux and cell wall breakdown.

Using gamma treatments caused many variants in color and shape of the flowers, compared with non-irradiated plants, besides, the high gamma doses of 40 and 60 Gy recorded the highest number of changes. In this respect, (Kaur *et al*., 2017) used gamma radiation on *Calendula officinalis* seeds at 20, 40, 60, 80 and 100 Gy. In comparison to the control, all doses induced flowers abnormalities, in color, form and shape, plants, (Li *et al*., 2022) on *Tulipa gesneriana* applied gamma radiation at 0, 5, 10, 20, 40, 60, 80 and 100 Gy. They recorded four different flower color variants, and (El-Khateeb *et al*., 2023) mutated *Gaillardia pulchella* seeds with sex doses of gamma radiation (10–60 Gy). They recorded many morphological changes in color and shape of the flowers. 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

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recessive mutations as identified by (Dowrick and Bayoui, 1996) on chrysanthemum plant. (Mato *et al*., 2000) stated that anthocyanin accumulation in different flower colors occurred during the blockage at the early and late stages of anthocyanin production.

Also, data showed that, the low doses of 20 followed by 10 Gy improved anatomical structure of the leaves and flowers and induced changes in cells number and it size compared to the control. Such results are similar to those gained by (Sakr *et al*., 2013) on *Dracaena surculosa* plant, used gamma irradiation at 0, 5, 10, and 15 Gy, they found that the dose of 10 Gy, gave the greatest diameter, epidermis thickness, cortical thickness, and vascular bundles dimensions (mm) of the stem and root; (Eid *et al*., 2021) treated *Helichrysum bracteatum* seeds with gamma doses from (5- 40 Gy), the low dose of 15 Gy produced largest midveine (mm), largest bundle dimension, the thickest lamina, widest diameters of flowering bud and receptacle (mm). In this respect, 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. Therefore, these changes in anatomical structure, will make it possible to deduce how cells are damaged by and protect against gamma irradiation in the plants, and thereby provide critical keys for understanding the effects of gamma irradiation on tissues and cells of the plants.

Moreover, (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 increase in the number of branches and leaves; (Pitirmovae, 1979) reported that gamma radiation caused changes in metabolic processes that affect phytohormone growth or nucleic acid synthesis; (Dickison, 2000) stated that the anatomical structure of the leaf can be change as a result to gamma radiation that is ionizing 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; (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, and (Harahap, 2005) 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.

CONCLUSION

Gamma irradiation provides a feasible choice to plant breeders for bringing desired traits concerned with better germination and plants' growth thus avoiding high throughputs of time, labor and cost generally related with selective breeding methods. Since these traits are controlled by genes, positive fluctuation in genomic structure of plants of interest by exposure to gamma irradiation can enhance their germination and general growth characters. It is evident from the surveyed literature that in many instances, low doses of gamma irradiation promote germination and growth characters of different plants while higher doses have detrimental influences. Lower doses stimulate the growth of subject plant by either direct genome modifications or regulation of cellular process which include hormonal signaling, enhancing enzymatic efficiency, increasing anti -oxidative potentials, cell membranes modification etc. which could lead efficient cell division, high photosynthesis rate and improved capacity of plants to cope with environmental stresses. Conversely, higher doses cause genomic damage, production of free radicals and reactive oxygen species which influence germination and growth factors in a negative manner resulting in arrested germination, survival and growth abnormalities. Although the optimum dose of gamma irradiation for growth stimulation is difficult to suggest for mutation breeding since different plants respond differentially to applied doses; however, it seems that in most studies radiation low doses correspond to enhanced germination and growth performance of many plant species.

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