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Research Article
In vitro Mutagenesis Induction in *Eustoma grandiflorum* Plant using Gamma Radiation

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Abstract

Background and Objective: Mutagenesis is the process in which genetic information in an organism is changed, not by genetic segregation but induced by chemical or physical agents. The experiment was conducted to investigate the effects of gamma irradiation as physical mutagens on *E. grandiflorum* plant. **Materials and Methods:** The experimental study was carried on *Eustoma grandiflorum* plant to evaluate the effects of gamma radiation as physical mutagenesis at different doses (0, 2, 5, 10, 20, 40, 60, 80, 100 and 120 KR) using Co^{60} gamma source for 30 min on various morphological, flowering and anatomical characters by Duncan's New Multiple Range test at 0.05% level of probability. **Results:** *In vitro* shooting and rooting behaviors showed the best results with low gamma irradiation (10 KR), while the longest shoots and the highest number of leaves were obtained with gamma dose 60 KR. The highest number of roots and the longest roots were observed with 20 KR of gamma radiation. Highest survival percent of acclimatized plants (63%) was obtained with 10 and 20 KR treatments. A wide range of leafs and flowers colour and form changes were observed after radiation with different doses of gamma rays. Different leaf anatomically structure features that depend on the level of gamma radiation treatments were recorded. Total proteins were extracted from leaves after flowering and analyzed by SDS-PAGE and revealed 26 protein bands with 100 KR and absence with other doses or control. **Conclusion:** It was concluded that, Gamma radiation with different doses had a potential effect on the production of mutant *E. grandiflorum* plants due to changing in flowers colour and form.

Key words: Lisianthus, *in vitro* mutagenesis, gamma radiation, total proteins, extraction

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Lisianthus (*Eustoma grandiflorum*) is an annual or perennial plant, belongs to the Gentianaceae, ranked in the top 10 cut flowers worldwide due to its rose-like appearance and its variability in various colours[1]. Recently, a variety of cultivars had been developed with many traits such as flowering throughout the year, heat tolerance, flower colour, flower size and form[2]. Its popularity should continue to increase throughout the next century because the flower possess the qualities of an ideal cut flower.

The common propagation of *E. grandiflorum* is by seed or cutting but the quality is not uniform due to the variations in flowering time, plant height and the number of flowers. Studies dealing with the propagation of *E. grandiflorum* by tissue culture technique is relatively low. Some studies on micro propagation of *E. grandiflorum* have been revealed[3-8]. Several factors like genotype, media, plant growth regulators and type of explants should affect the success of the micro propagation method, most of plant growth regulators that have been used were 6-Benzyladenine, Kinetin, naphthalene acetic acid and Indole-3-butyric acid [9,10].

Mutagenesis increase the possibilities of variability creation with high ornamentation[11]. Mutation is a natural process which creates changes in DNA sequences. The genetic variation created is useful because it helps population to survival and change over time, which can be induced by chemical and physical agents[12].

Gamma irradiation is a physical mutagenesis. It can be used for irradiating whole plants and delicate materials, such as pollen grains. It has a shorter wave length and therefore, possesses more energy than protons and X-ray, which gives them ability to penetrate deeper into the tissue[13]. Abdullah *et al.*[14] mentioned that mutation induction in ornamental plants with gamma irradiation have been used for genetic changes, high flower yield, disease resistance, early maturity. Gamma rays are known to influence plant growth and development by inducing cytological, genetical, biochemical, physiological and morphogenetic changes in cells and tissues. These effects after exposure were deeply influenced by several factors, some of these factors are released to plant characteristics such as species, cultivar and stage of development and other related to radiation features as quality, dose and duration of exposure[15].

Determination of protein molecular weight via SDS-PAGE is a universally used method in biomedical research. Also, it can be economically used to assess genetic variation to differentiate mutants from their parent genotypess[16]. Electrophoretic analysis of protein provides information concerning the structural genes and their regulatory systems that control the biosynthetic pathways of that protein. Each polypeptide band represents the final products of transcriptional events occurring due to active structural genes[17].

The objective of the present study was to study the effect of various doses of gamma radiations as physical mutagens on *E. grandiflorum* propagated *in vitro* as well as flowering of acclimatized plants, biochemical and cytological behaviors.

MATERIALS AND METHODS

The experiment was conducted from 3/2014-12/2016 at Ornamental Plants Department, Faculty of Agriculture, Cairo University and Tissue Culture Laboratories of Ornamental Plants and Woody Trees and Biotechnology Departments, National Research Centre (NRC), Egypt.

Plant materials and surface sterilization: *E. grandiflorum* plants were obtained from greenhouse of National Research Centre on 1/3/2014 and prepared by washing lateral buds as explants under running tap water and a few drops of hand washing liquid for 20 min. After 3 times rinses with distilled water, explants were surface sterilized in 70% (v/v) ethanol for 1min. After that, 20% commercial sodium hypochlorite solution and one drop of tween 20 for 10 min were used then rinsed three times with autoclaved distilled water followed with 7 min in 0.1g L^{-1} $HgCl_2$. Finally, the explants were rinsed 3 times with autoclaved distilled water.

Culture media and culture conditions: The explants were cultured on MS medium (free hormones) supplemented with 25 g L^{-1} sucrose and 8 g L^{-1} agar. The culture medium was adjusted to pH 5.6±0.2, autoclaved at 121°C and 1.5 kg cm^{-2}. The cultures were incubated under 30 umol m^{-2} sec^{-1} of light and 16 h photoperiod. After one month from culture explants, the shootlet nodal stems were used for *in vitro* propagation.

Proliferation of shootlet explants: Shootlet nodal stems were cultured on medium supplemented with different cytokines [6-benzyl amino purine (BA), 6-γ, γ-dimethylallylaminopurine riboside (2ip) and Kinetin (N6fouryla denine) (Kin)] at concentration of 0.4 mg L^{-1}. The obtained shoots were repeatedly sub-cultured and the mean of 2 subcultures data was calculated. Characters including

shoot number shoot length (mm.) and number of leaves formed per shootlet after 45 days from each subculture under control and cytokines treatments.

Gamma irradiation as physical mutagenesis: Gamma irradiation experiment was carried out at Middle Eastern Regional Radioisotope Centre, Dokki and Giza, Egypt. After culturing the explants on MS medium free hormones, shootlet explants were subjected to various doses of gamma radiation (0, 2, 5, 10, 20, 40, 60, 80, 100 and 120 KR) using Co^{60} gamma source for 30 min. The irradiated shoots were incubated under the above mentioned conditions and subcultured 3 times, 4-5 weeks after 3rd subculture; the *in vitro* morphological characters were recorded: Number of shootlets/explant, shootlets length (cm), number of leaves/shootlet, rooting (%), number of roots/shootlet and root length (cm).

Acclimatization process: The *in vitro* rooted plants were successfully transplanted to the greenhouse of National Research Centre(17/2/2015) using growth media contained of perlite and peattomse (1:1), morphological characters (Survival percentage, number of branches, height of branches/ plant(cm), number of leaves/branch and leaf area (cm^2) were recorded after 2 months.

After 3-4 months from acclimatization process, flowering characters (Days to flower bud initiation, days to bloom, flowering percentage, number of flower buds/plant, number of flowers/plant, flower diameter (cm), bloom stem length (cm), peduncle length (cm), days to flower senescence (from blooming), number of petals/flower, petals area (cm^2), number of stamens, fresh and dry weights of flower (g) were recorded.

Analysis of protein profile of leaf by SDS-PAGE: Samples (0.5 g) were homogenized with 2 mL of a buffer containing 50 mm Tris (hydroxymethyl) amino methane (Tris)-Glycine (pH 8.3), 0.5 m sucrose, 50 mm EDTA, 0.1 m KCl, 2 mm PMSF and 0.1% (v/v) 2-mercaptoethanol in a chilled pestle and mortar at 4°C. The homogenate was centrifuged in a cooling centrifuge (Sigma, 2-16 PK, Germany) at 14,000×g for 10 min. Protein concentration in the supernatant samples was estimated according to the method of Bradford[18]. Supernatant samples (40 µg protein) were mixed with equal volumes of solubilizing buffer [62.5 mm Tris-HCl, pH 6.8, 20% (w/v) glycerol, 2% (w/v) SDS, 5% (V/V) 2 mercaptoethanol and 0.01% bromophenol blue] and heated for 4 min at 95°C, then cooled on ice before loading on 12.5% polyacrylamide[18].

Statistical analysis: The data were analyzed using randomized complete block design with 10 replicates per treatment. The treatments means were compared for significance by Duncan's New Multiple Range test at 0.05% level of probability[19] using COSTATV-63.

RESULTS AND DISCUSSIONS

Effects of cytokine type on shootlet proliferation: The tabulated data (Table 1) indicated differences *in vitro* shoot proliferation of *E. grandiflorum* plant as a result of using various cytokine types in MS culture medium. Data showed that both number of shootlets formed per explant and number of leaves per shootlet were in highest values (10.77 and 66.67, respectively) when BA was added to the culture medium at 0.4 mg L^{-1} as compared to control (MS free hormones) and other cytokines were used (2ip and Kin).While, the longest shoots (2.49 cm) were obtained with MS culture medium supplemented with Kin (0.4 mg L^{-1}). These results could be explained by cytokines have important role to stimulate cell division and cell elongation. This explained by its role in activating RNA synthesis and to stimulate protein synthesis as well as enzyme activity[20]. Results are in agreement with those obtained by Takayama *et al.*[21]. They noticed that BA was more effective for *Lilium bulblets* development. Also, Raad *et al.*[22] found that the better shoot proliferation in terms of shoot number was obtained using BA compared with kinetin. Moreover, indicated that when *Ficus Carica* explant was cultured on medium containing kinetin gave the longest shoots[23].

Gamma radiation effects on *E. grandiflorum* plant

***In vitro* shooting and rooting behavior:** It is evident from the data presented in Table 2 that irradiation the shootlets of *E. grandiflorum* effected the *in vitro* shooting and rooting behaviors. Gamma irradiation at 10 KR caused the highest number of shootlets formed per explant (12.5) as compared with control (un-irradiated shoots) and other treatments, while the longest shoots and the highest number of leaves (5.58 and 45.5 cm, respectively) were obtained when the shootlets were irradiated with gamma dose 60 KR. For *in vitro* rooting behavior, it is clear that, gamma irradiation of shootlets decreased the percentage of rooting except for the irradiation treatments at 10 and 20 KR which caused as highest (100%) rooting as control treatment. The highest number of roots and the longest roots (16.5 and 2.75 cm, respectively) were observed with 20 KR of gamma radiation. The results are in

Table 1: Effects of cytokinin type on *in vitro* shootlet proliferation of *Eustoma grandiflorum*

Characters/treatments	No. of shoot/explant	Shoot length (cm)	No. of leaves/shootlet
Control	0.663^{b}	1.99^{ab}	19.11^{b}
BA	10.77^{a}	0.99^{b}	66.67^{a}
2ip	1.77^{b}	2.16^{ab}	15.44^{b}
Kin	2.55^{b}	2.49^{a}	13.99^{b}

^{a,b}Means followed by different letters are significantly different, means with the similar letters in each column are not significantly different at 5% level of probability using Duncan's test

Table 2: Effects of gamma radiation on *in vitro* shooting and rooting behaviors of *Eustoma grandiflorum* plants

Characters /dose (KR)	No. of shootlets /explant	Length of shootlets (cm)	No. of leaves /shootlet	Rooting (%)	No. of roots /shoot	Length of roots (cm)
Control (0)	1.50^{c}	4.16^{ab}	29.33^{bc}	100^{a}	5.83^{c}	1.05^{bc}
2	1.67^{c}	4.67^{ab}	37.67^{ab}	50^{ab}	8.83^{bc}	1.05^{bc}
5	2.83^{c}	3.83^{ab}	31.16^{bc}	67^{ab}	4.16^{c}	1.016^{bc}
10	12.50^{a}	4.00^{ab}	26.67^{c}	100^{a}	13.67^{ab}	1.25^{bc}
20	5.67^{b}	3.75^{ab}	28.33^{bc}	100^{a}	16.50^{a}	2.75a
40	2.00^{c}	5.25^{ab}	28.83^{bc}	83^{ab}	8.16^{bc}	2.12^{ab}
60	2.50^{c}	5.58^{a}	45.50^{a}	67^{ab}	6.75^{bc}	2.12^{ab}
80	1.83^{c}	5.41^{a}	29.67^{bc}	83^{ab}	4.25^{c}	1.33^{bc}
100	1.83^{c}	4.25^{ab}	36.33^{abc}	67^{ab}	4.50^{c}	0.61^{bc}
120	0.75^{c}	2.91^{b}	28.16^{bc}	50^{b}	3.00^{c}	0.32^{c}

^{a,b,c}Means followed by different letters are significantly different, means with the similar letters in each column are not significantly different at 5% level of probability using Duncan's test

agreement with[24] on *Hibiscus rosasinensis*, mentioned that application of low doses of gamma rays resulted in the highest number of shootlets and the maximum number of leaves/explant. In this study, the increase in doses of gamma rays to 120 KR caused decrease in all characters in both shoots and roots *in vitro*. This inhibitory effects of gamma radiation at high doses on *in vitro* characters may attribute to disturbances of auxin synthesis[25].

Gamma radiation effects on morphological characters of developed plants after adaptation process: Data presented in Table 3 indicates that gamma radiation at low doses had stimulation effect on survival and morphological characters of adapted *E. grandiflorum* plant except for the dose 2 KR which had inhibit effects on the most morphological and floral characters (caused non flowering) of adapted plants as compared to control. Highest survival percent of plants (63%) was obtained with 10 and 20 KR treatments, this percent was decreased significantly (at 5% level of probability) with increasing the dose of gamma radiation. Similar results were found on *Chrysanthemum morifolium* by Banerji and Datta[26]. Branches number and height as well as number of leaves/branch were in highest values (3.33, 11.67 and 35.33 cm, respectively) with 20 KR dose of gamma radiation. The highest value of leaf area (5.28 cm^2) was obtained with 10 KR dose treatment. Confirmed results were observed by Dilta *et al.*[27] in plant height after 2 KR gamma rays exposure of chrysanthemum varieties. From these results we can observe the reduction in the size of leaves with increase in gamma radiation dose above 10 KR. Moreover, change of the color of leaves (pied color) was observed (Fig. 1) with increasing

Fig.1: Pied color leaves of adapted *Eustoma grandiflorum* irradiated with gamma rays at 100 KR

Table 3: Effects of gamma radiation on morphological characters of *Eustoma grandiflorum* adapted plants

Characters /dose (KR)	Survival (%)	No. of branches	Height of branches /plant (cm)	No. of leaves /branch	Leaf area (cm^2)
Control	21.6^{c}	1.33^{c}	1.30^{b}	16.00^{d}	1.490^{c}
2	41^{b}	1.33^{c}	1.83^{b}	18.67^{cd}	4.074^{ab}
5	45^{b}	1.33^{c}	3.33^{b}	24.00^{bcd}	2.709^{bc}
10	63^{a}	2.67^{ab}	1.067^{b}	14.33^{d}	5.283^{a}
20	63^{a}	3.33^{a}	11.67^{a}	35.33^{a}	4.040^{ab}
40	45^{b}	2.00^{ab}	9.67^{a}	29.00^{abc}	3.620^{ab}
60	37^{b}	1.33^{c}	3.16^{b}	28.67^{abc}	3.538^{ab}
80	37^{b}	1.33^{c}	4.50^{b}	31.67^{ab}	2.593^{bc}
100	37^{b}	1.00^{c}	1.83^{b}	22.33^{bcd}	2.807^{bc}
120	37^{b}	1.00^{c}	1.43^{b}	18.33^{cd}	2.660^{bc}

^{a,b,c,d}Means followed by different letters are significantly different, means with the similar letters in each column are not significantly different at 5% level of probability using Duncan's test

Table 4: Effects of gamma radiation on floral characters of *Eustoma grandiflorum* adapted plants

Dose(KR)/characters	Control (0)	5	10	20	40	60	80	100	120
Days to flower bud initiation	107^{b}	111^{b}	176.67^{a}	130.5^{ab}	129^{ab}	145.83^{ab}	101.67^{b}	136^{ab}	95^{b}
Days to bloom	118^{bc}	131^{bc}	187.67^{a}	141.0^{abc}	137.33^{abc}	157.83^{ab}	117.5^{bc}	143.67^{abc}	101.67^{c}
Flowering percentage (%)	18.516^{b}	18.516^{b}	9.77^{b}	11.11^{b}	38.99^{a}	10.44^{b}	18.516^{b}	38.99^{a}	35.29^{a}
No .of flower buds/plant	1^{b}	2^{b}	1^{b}	2.67^{b}	1.33^{b}	3.67^{b}	7.67^{a}	3.67^{b}	2.67^{b}
No. of flowers/plant	1.67^{ab}	2.67^{ab}	1^{b}	4.00^{ab}	1.67^{ab}	6.00^{ab}	7.00^{a}	3.33^{ab}	7.00^{a}
Flower Diameter (cm)	1.67^{c}	3.43^{ab}	2^{bc}	3.26^{abc}	4.30^{a}	2.33^{bc}	3.6^{ab}	4.3^{a}	4.8^{a}
Bloom stem length (cm)	6.5^{b}	5.83^{bc}	2^{c}	5.33^{bc}	6.67^{b}	7.33^{ab}	8.67^{abc}	9.16^{ab}	11.16^{a}
Peduncle length (cm)	3.67^{cd}	5.67^{abc}	2^{d}	5.83^{abc}	7.46^{ab}	3.5^{cd}	5.5^{bc}	8.67^{a}	6.83^{ab}
Days to flower senescence (from blooming)	4.33^{bc}	7.33^{abc}	3.67^{c}	6.67^{abc}	5.67^{abc}	9.33^{ab}	6.67^{abc}	10^{a}	10^{a}
No. of Petals/flower	11^{bc}	12.33^{abc}	9.00^{c}	12^{bc}	15.33^{ab}	15.67^{ab}	12^{bc}	18^{a}	18.67^{a}
Petals area (cm^2)	3.18^{cd}	3.83^{bcd}	2.17^{d}	3.18^{cd}	5.53^{b}	2.36^{d}	5.06^{bc}	5.01^{bc}	8.77^{a}
No. of Stamens	5.33^{a}	4.33^{a}	3.33^{a}	5.33^{a}	4.33^{a}	3.00^{a}	4.33^{a}	5.67^{a}	5.00^{a}
F.W. of flower (g)	0.61^{ab}	0.27^{bc}	0.013^{c}	0.27^{bc}	0.69^{ab}	0.11^{bc}	0.43^{bc}	0.69^{ab}	1.16^{a}
D.W. of Flower (g)	0.095^{b}	0.083^{bc}	0.006^{d}	0.06^{bcd}	0.092^{bc}	0.018^{cd}	0.063^{bcd}	0.092^{bc}	0.20^{a}

^{a,b,c,d}Means followed by different letters are significantly different, means with the similar letters in each column are not significantly different at 5% level of probability using Duncan's test

gamma radiation dose to 100 KR. This may attributed to the poor growth of plant due to radiation damage of the irradiated plants particularly chromosomal breakage[28]. Zargar *et al.*[29] pointed out that gamma rays significantly decreased (at 5% level of probability) leaf length and width of Chrysanthemum varieties.

Gamma radiation effects on floral characters: The effects of gamma radiation on floral characters were observed as a result of different gamma radiation doses (Table 4). The result showed that the irradiated plants at 10KR dose delayed both flower bud initiation and bloom formation (176.67 and 187.67 days, respectively), whereas the earliest ones (107 and 118) and (95 and 101.67 days) were observed for un-irradiated plants and 120 KR dose of gamma radiation, respectively. It seems that all doses of gamma radiation except 120 KR dose had inhibit effect on bud and bloom formation, this may attributed to alterations in the rate of physiological processes with gamma radiations. These results are confirmed by some researchers[30,31], reported that mutagenic agents such as gamma rays reduce viability and sprouting speed. Mahure *et al.*[32] mentioned that the biosynthetic pathways are changed with radiation which are directly and indirectly associated with physiological processes of flowering. Recently, Navabi *et al.*[33] revealed that, dose treatment of 10 Gy delayed time to sprout but all plants irradiated at doses of 50 and 100 Gy, 57, 29% of plants were sprouted and grew, respectively. It can also notice from data in Table 4 that the highest percentage of flowering (35.2938.99%) and the biggest flower diameter (4.3-4.8 cm) were obtained with 40, 100 and 120 KR doses of gamma radiation. The highest number of flower buds formed per plant (7.67) was found with 80 KR, meanwhile increasing this dose up to 100 or 120 KR increased the number of flowers/plant and bloom stem length but the longest peduncle was observed with 40 and 100 KR doses of gamma radiation. Contrary, Kumari *et al.*[34] on *C. morifolium* observed that the increase in doses of gamma rays had significantly (at 5% level of probability) reduced the number of flower heads per plant; the maximum flower head size was recorded with untreated plants.

Fig. 2(a-h): Changes in *Eustoma grandiflorum* flowers colour and form after radiation with different doses of gamma rays (a) Control (untreated plants), (b) 5 KR, (c) 20 KR, (d) 40 KR, (e) 60 KR, (f) 80 KR, (g) 100 KR and (h) 120 KR

In this study, using high doses of gamma radiation (100 or 120 KR) delayed the flower senescence to 10 days and caused the highest values of petals number and flower fresh and dry weights. While, untreated plants and 10 KR led to earlier flower senescence (4.33 and 3.67 days, respectively) and reduced the above mentioned characters. The finding by Navabi *et al.*[33] also reported that the dose of 100 gamma ray caused a significant reduction in the number of floret petals. The results showed that all doses of gamma radiation had not significant effect on the number of stamens per flower. The highest values of fresh and dry weights of flower were observed with 100 KR dose of gamma radiation.

Changes in flowers color and form after radiation with different doses of gamma rays: The results showed a wide range of flower color and form Fig. 2 as following: The flowers of untreated plants (Fig. 2a) are light purple mid-petalled scheme by white color with dark purple center of the flower. In the radiation dose 5 KR (Fig. 2b), the flower light/dark purple without the white color in the middle of the petals, the number of rows of petals few with small wide but wrapped. About 20 KR dose of gamma radiation (Fig. 2c) caused very light mauve flowers scheme by light white, middle of the flower dark purple serrated edge. About 40 KR dose (Fig. 2d) produced a very dark purple flower with mosaic white on the external petals. About 60 KR dose (Fig. 2e) resulted very light purple flowers yellowish with white color from abroad. In 80 KR dose (Fig. 2f), very dark purple petals mid-pied by white, center of the flower is dark purple. In 100 KR dose (Fig. 2g), mauve petals scheme by white middle/center of the flower dark purple. However, 120 KR (Fig. 2h) of gamma radiation resulted in interest change in flower color, light purple. Second flower on the same plant is dark purple and there is a halo of red color in the middle of the petals which wrapped around each other (integrated) takes a narrow oval shape. The change in flower form as result of gamma ray was also marked by on *C. morifolium* and *C. morifolium* who attributed the abnormality flower forms to chromosomal aberrations, disturbance in the production and/or distribution of growth substances caused by gamma rays[34,35].

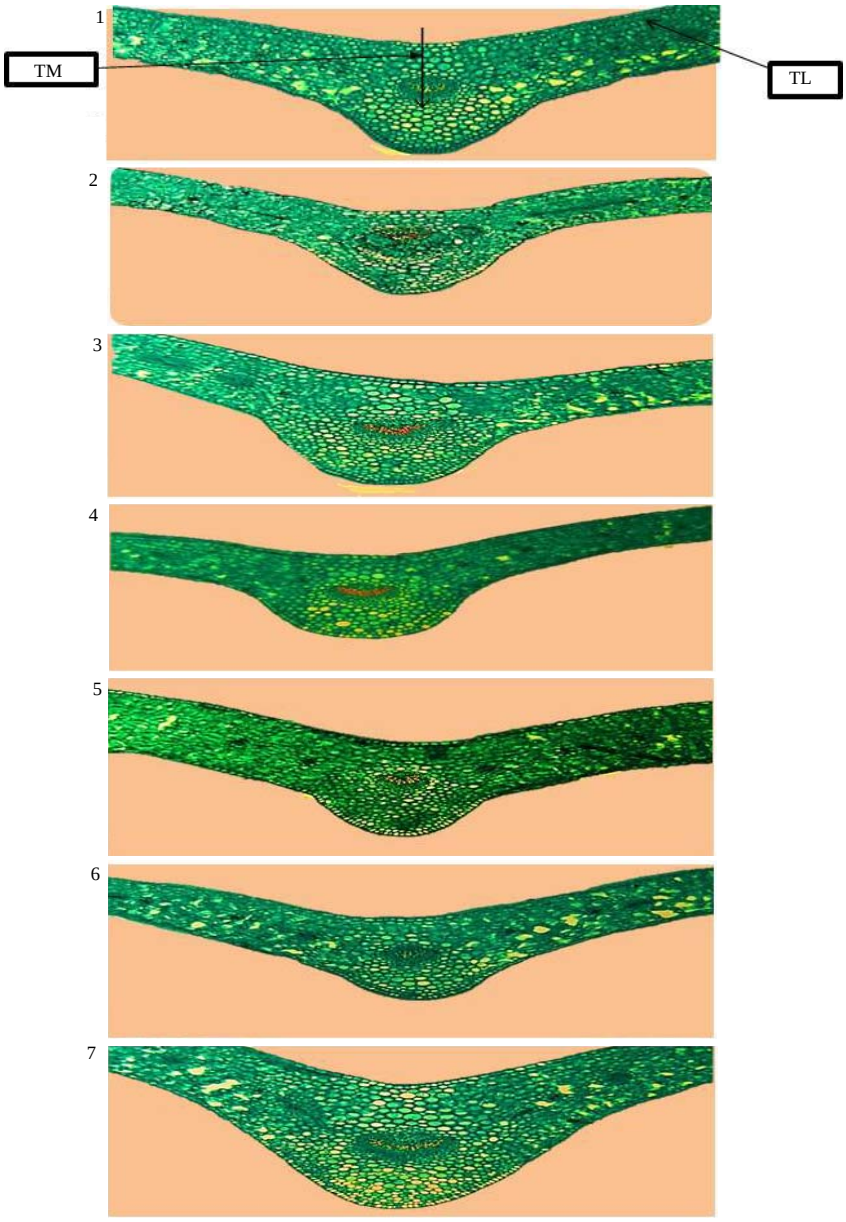

Fig. 3(1-7): Light microphotograph showing transverse sections through the blade of the third *in vitro* plant leaf developed on main stem of *Eustoma grandiflorum* plantlets. (x10)(Bar: 0.05 mL). TM: Thickness of Midvein and TL: Thickness of Lamina

Table 5: Effects of gamma radiation on leaf anatomy of *Eustoma grandiflorum* adapted plants

Dose(KR) /characters	Thickness of midvein (µ)	Thickness of lamina (µ)	No. of xylem rows	No. of vessels	Length of vascular bundle (µ)	Wide of vascular bundle (µ)
Control	105	70	13	44	32	28
5	107.5	50	9	33	20	19
20	130	68	18	65	48	30
40	100	58	20	65	40	29
80	105	68	12	37	30	27
100	95	76	10	22	29	20
120	145	110	21	82	43	38.5

Gamma radiation effects on anatomical characters: As shown in Table 5 and Fig. 3 and 4, the highest values of thickness of midvein, thickness of lamina, number of xylem row, number of vessels and wide of vascular bundle were

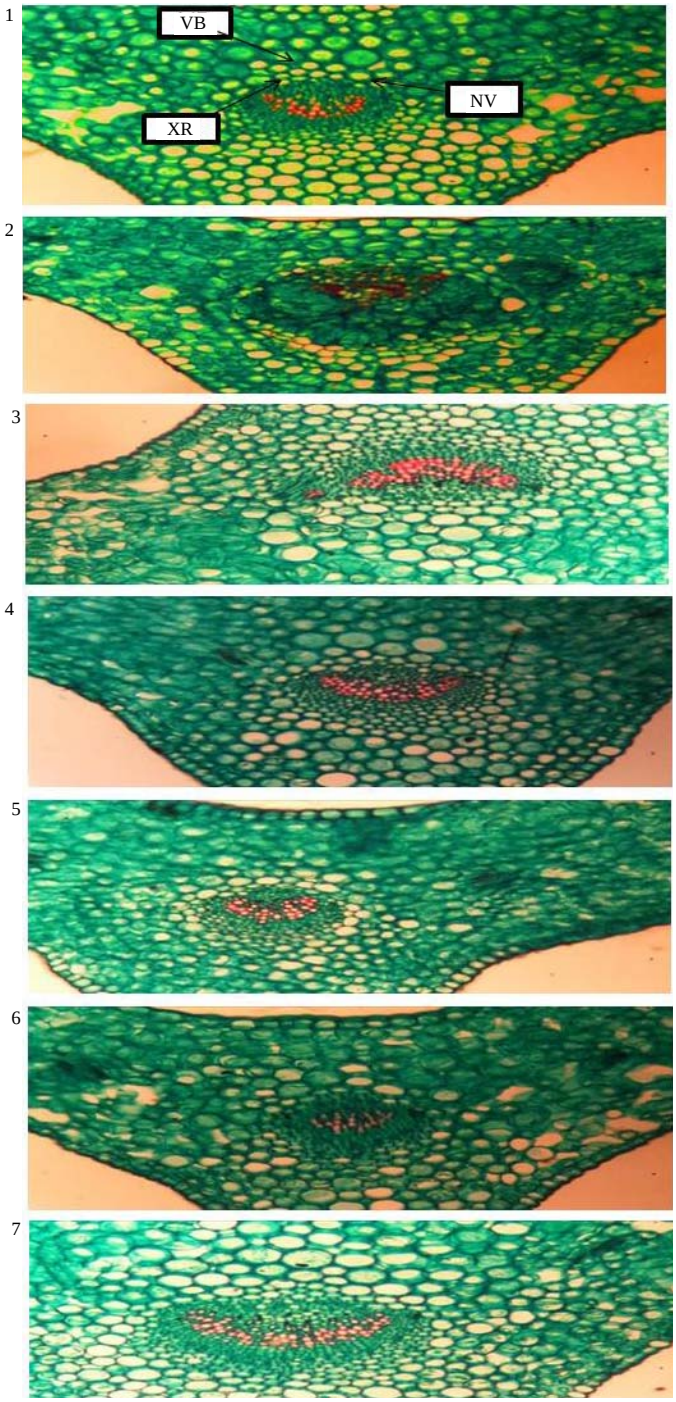

Fig. 4 (1-7): Light microphotograph showing transverse section through the blade of the third *in vitro* plant leaf developed on the main stem of *Eustoma grandiflorum* plantlets. The section shows vascular bundle, (number of vessels and number of xylem rows. (x40)(Bar: 0.05 mL), VB: Vascular bundle, XR: Xylem rows and NV: No. of vessels

produced by gamma rays 120 KR. While 20 KR of gamma ray recorded the highest values in length of vascular bundle followed by 120 KR of gamma ray compared to control plants and the other treatments. On the contrary, the decrease in all of previous anatomical structure characteristics were recorded by plants treated with dose 100 and 5 KR gamma compared to control. Except thickness of lamina recorded as light increase comparing with control plants .With respect to

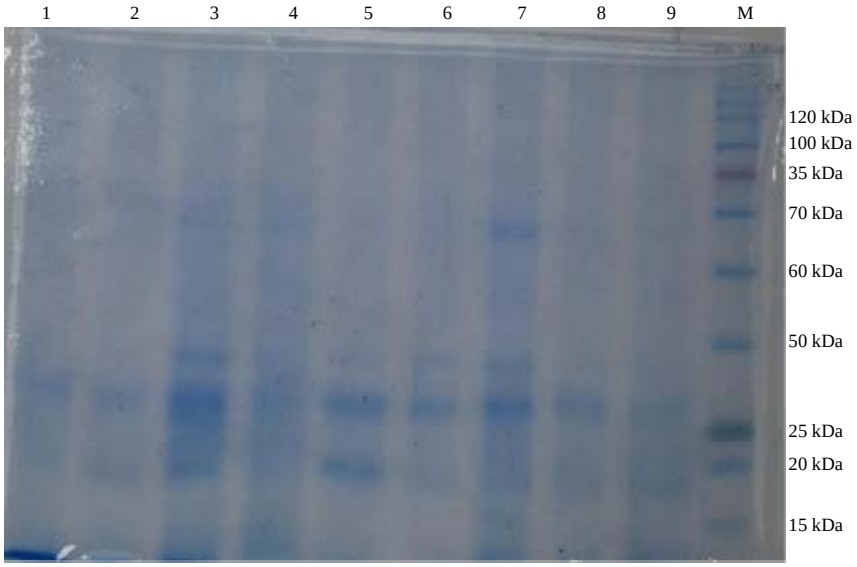

Fig. 5: Patterns of SDS-PAGE electrophoretic protein of *E. grandiflorum* plants respond to different doses of gamma irradiation. 1: 5 KR, 2: 10 KR, 3: 20 KR, 4: 40 KR, 5: 60 KR, 6: 80 KR, 7: 100 KR, 8: 120 KR, 9: Control and 10: Protein marker

application of gamma radiation treatments, data in Table 5 showed increments in thickness of midvein, the highest value was obtained by treated plants with dose 120 and 20 KR gamma rays. While, the highest value in thickness of lamina was recorded by doses 120 and 100 KR gamma rays. Gamma rays at 20 KR dose recorded the highest value in vascular bundle length compared to control and the other treatments. The data presented in this investigation indicates that gamma radiation treatments led to increase or decrease in thickness of midvein, thickness of lamina, number of xylem row and number of vessels, length of vascular bundle and wide of vascular bundle. These effects on anatomical parameters of *E. grandiflorum*, confirm that gamma rays has a promotive effect on some enzyme activity which are usually associated with certain metabolism and anabolic processes which affected directly or indirectly on growth and anatomical structure[36,37]. Gamma rays are ionizing radiation produce free radicals in cells which can damage or modify important components of plant cell. These modify reported to affect differentially the morphology and anatomy of plants depending on the irradiation doses and dilation of thylakoid membrane[37-39].

Gamma radiation effects on protein fractions: Total proteins were extracted from fresh leaves of control and different dosages gamma irradiated *E. grandiflorum* plants after flowering and analyzed by SDS-PAGE. These protein alterations based on changes in polypeptides Molecular Weights (MWs). The SDS-PAGE analysis revealed total of 11 polypeptides bands with different bands MWs that ranged from 66-17 kDa as shown in Fig. 5. Out of those 12 polypeptides bands, 2 common bands with all dosages of gamma irradiation and the control at MWs 27 and 21 kDa were appeared. SDS-PAGE generated 7 bands, which disappeared with the non-irradiated plant (control) and appeared with gamma irradiated plants. Gamma irradiation at 100 KR appeared one unique band at 55 kDa, which doesn't appear with other gamma dosages or the control one. The maximum number of bands (10 bands) were found with 100 KR gamma irradiation. While, the minimum number of bands (2 bands) were found with 5 KR of gamma irradiation. The present study observed that SDS-PAGE analysis of gamma irradiated and non-irradiated *E. grandiflorum* plants are based on variations in number of polypeptide bands and molecular weights of polypeptides bands as well as gain or loss of protein bands. The most of the polypeptide bands are of low molecular weights, this results are in agreement with Mandal and Datta[40]. They observed the SDS-PAGE analysis of *Corchorus olitorius* L. exposed to gamma irradiation, the most of protein bands are of low molecular weights. *Morus alba* variety S_{13} irradiated with different dosages of gamma irradiation, revealed the significant and major protein polypeptide with low molecular weight[41].

CONCLUSION

Gamma radiation at different doses can be beneficial for mutagenesis induction in *Eustoma grandiflorum* plant due

to changing in leaves and flowers colour and form. Low gamma irradiation (10 KR) showed the best results in *in vitro* behaviors of shooting and rooting as well as the survival percent of acclimatized plants:

- A wide range of leafs and flowers colour and form changes were observed after radiation with different doses of gamma rays
- Different leaf anatomically structure features that depend on the level of gamma radiation treatments were recorded
- Total proteins were extracted from leaves after flowering and analyzed by SDS-PAGE and revealed 26 protein bands with 100 KR and absence with other doses or control

SIGNIFICANCE STATEMENTS

This study discovers the possible synergistic effects of gamma radiation that can be beneficial for mutagenesis induction in *Eustoma grandiflorum* plant. This study will help the researcher to uncover the critical changes that many researchers were not able to explore. Thus, a new theory on these physical mutagenesis possibly at different doses, may be arrived at flowers trade markets.

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