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EFFECT OF MUTATION INDUCED BY GAMMA-IRRADIATION IN ORNAMENTAL PLANT LILIUM (*LILIUM LONGIFLORUM* CV. TRESOR)

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Abstract

Genetic diversity and its utilization in plant breeding is the main objective. Induction of Mutation has known as the major pathway for the induction of genetic diversity in flowers. In order to study the effect of gamma rays on the mutagenicity of lily cut flower a project was carried out in a completely randomized design with tree replications. Lilium bulbs were irradiated with six different levels of gamma rays (control, 10, 20, 30, 40 and 50 Gy). Then the number of leaves, leaf area, fresh and dry weight, stem height, leaf relative water content, ion leakage, chlorophyll a and b and total chlorophyll were measured. Irradiation results showed a significant difference in leaf number, ion leakage and chlorophyll a, b and total. In this GR_{50} most mutations occur in the sample and the damage will be minimal (growth reduction by 50%). Due to high coefficient of determination (R^2) obtained in dry weight; this factor is used to determine the GR_{50} compared with other characters is more suitable. The optimal dose of radiation at the plant was computed between 22.5-27.5 Gy. The consequence of treatment on leaf relative water content was not significant. In general High-gamma irradiation doses had harmful effects on growth of Lilium.

Key word: Balbus plant, Mutagenesis, Morphology, Optimum dosage**.**

Introduction

Lilium is related to the family of Liliaceae which is the famous ornamental flowers that are used over a wide area, after, roses, cloves, and chrysanthemums (Ko *et al*., 2002). The genus Lilium is an important genus from phylogenetical and horticultural points of view includes about 110 species, which are belonged to moderate regions of the north of hemisphere with a cold weather. Most of ornamentals, hybrid flowers and lots of Lilium species are cultivated because of their esthetic value (Du *et al*., 2017). Todays this is particularly relevant where the modern floriculture industry is always seeking novel characters to surprise the market and there is always interest and need for novel ornamental crop species. Characters of interest are new flower/leaf colors and morphology and long flower shelf-life to meet the continuous demand of commercial growers and consumers for value-added varieties (Zaiton *et al*., 2012). From consumable point of view by a technology which its application is relatively easy, in both *In vivo* and *In vitro* systems, modern traits in ornamentals form, leaf and petal color and shape can be induced without a negative, residual effect on human health (Datta & Teixeira da Silva, 2006). One of the greatest goals in developing ornamental industry is producing flower with exotic petal shapes and colors and usually flower color is an important customer-friendly feature in greenhouse products, so breeders are always looking for a new color. In this case a lot of methods such as Breeding by hybridization or mutation (Okitsu *et al*., 2018) or different chemicals (Barakat & El-Sammak, 2011) have been used successfully to produce new flower color and shape. Mutagen induction is a common technology for improving plant genetically and phenotypically, and the drill has been effectively used to produce some modern cultivars in both horticulture and floriculture generally by improving existing cultivars (Simsek, 2010; Suprasanna *et al*., 2015). Spontaneous variations occur in ornamentals with a low level, which is usually referred to as Sports or somatic mutation. Therefore, by using breeding methods through mutagenesis, new cultivars can be created with favorable genetic variation of flower consumers, which require growth conditions such as wild cultivars as preference of consumers, are shapes and colors of wild flowers (Schum, 2003; Simsek *et al.*, 2010). The meaning of induced variation for plant betterment, by application of irradiation is related to the beginning of the 20^{th} century. In this way, plant genome is changed by irradiating some parts of plant with physico-chemical alterations (Datta & Teixeira da Silva, 2006). The methods for inducing novel shapes and improving flowers are unlimited, and induced variation help the breeder to improve a plant by mutagenesis. Kovács & Keresztes (2002) demonstrated the damaging biological effect that ionizing radiation has on plant cells, and the manners in which plant cells compensate for this damage. A lot of novel colour/shape of ornamentals has been produces successfully by using of irradiation technique. About 70% of mutated varieties are produced by gamma rays (Khatri *et al.*, 2005). There are two pathways for gamma rays, in the field (chronic) or in the laboratory (acute) (Nagatomi & Degi, 2009). Therefore, irradiation with the least damage is considered. Walther & Sauer (1986) determined radiosensitivity and reported that 20 Gy X- or γ-rays as the optimum dose for *In vitro* mutagenesis in gerbera and rose. The use of γ-rays is a common and safe practice in the inhibition of potato sprouting globally (Kameyama & Ito, 2000) and also flower mutagenesis (Kondo *et al*., 2009). Yadave (2016) exposed the plant of *Canscora decurrens* to γ-rays at different rates (5, 10, 15, 20, 25, 30, 35, 40, 45 and 50kR). The levels of 10, 15, 20 and 25kR radiations cause to induction mutagenesis in the length of plant, number of nodes and leaves and also fresh and dry weight, whereas the mentioned traits were decreased in 30, 35, 40, 45 and 50kR levels of irradiation. In the other experiment the effect of various levels of γ-rays on some traits of different varieties of sunflower were evaluated. Results showed that increasing in radiation levels has no effect on the present of seed germination. However, as well as increasing in gamma radiation levels seedling height were decreased. The levels of 148 Gy and 165 Gy gamma radiations cause to decrease in growth by 50 percent (GR_{50}) for AS508 and Nantio, respectively (Yalcin & Ulakoglu, 2019). Mutants with changed flower colour and low light tolerant have been induced by treating bulb scale of different Lilium hybrids (Broertjes & Alkema, 1970; Shah & Rahman, 2009) while Iizuka & Ikeda (1968) studied effects of X-ray on different characters of *Lilium formosanum*. In the other study the performance of different rates (0.0, 0.5, 1.0, 1.5, 2.0, and 2.5 Gy) of γ-rays on adventitious bud formation from bulblets were investigated. Data implies that the optimal radiation level was 1.0 Gy. Treatment with 1.0 Gy gamma ray cause to 55.33% survival in treated bulblets and 39.27% variation rate *In vitro* conditions. On the other hand, as well as increase in radiation level, the ability to forming the adventitious buds were decreased significantly (Xi *et al*., 2012). Aslam *et al*., (2016) exposed lilium bulbs with different levels of gamma ray. Results showed that irradiated liliums were significantly different in morphological characteristics compare controls.

The dose ratio used for mutagenesis is a major parameter in irradiation experiments and its effects have been evaluated by survey of various traits including mortality, growth and fertility. On the other hand variations in response to radiation among varieties of the species of the same genus are considerable (Pershad & Bowen, 1961). So in the present study the variability induced by optimum dose of gamma-irradiation in *Lilium longiflorum* cv. Tresor and screens plants with some variations in vegetative aspects and rate of survival were subjected.

Material and Methods

Plant material: F1 hybrid Bulbs of Asiatic lily (*Lilium longiflorum* cv. Tresor) purchased from a Company importing bulbs of ornamental plants were used for irradiation. Bulbs were washed and kept in hemp sacks for 48h in a cool and dark place to prevent of weight loss. Then bulbs placed in BB13-5 container (which result in dose rate of 15.7 Gy/min) and irradiated at 6 levels of gamma ray (0, 10, 20, 30, 40 and 50 Gy) with 3 replications by application of gamma cell irradiator at agricultural and nuclear medicine research center, Tehran, Iran. Gamma rays were generated from Cobalt-60. Irradiated flower bulbs were immediately planted in a growing medium containing peat, perlite and sand (8:8:1 v/v). We used of 1/2 Hoagland solution every 3 days for irrigation. Better growing conditions foe liliums are achieved in greenhouses by maintaining 24±4°C with 60-70 percent humidity (Fig. 1).Then some of morpho-physiologycal traits of flowers were monitored and recorded after completing of the plant growth period. After measurement all bulbs were planted back into single pot in well-drained soil and data on survival will recorded until 11 weeks.

Method of analysis: After experiment some of morpho-physiological parameters of lilium flowers were measured as below;

Number of leaves: For evaluating the plant growth we counted the number of leaves per each stem.

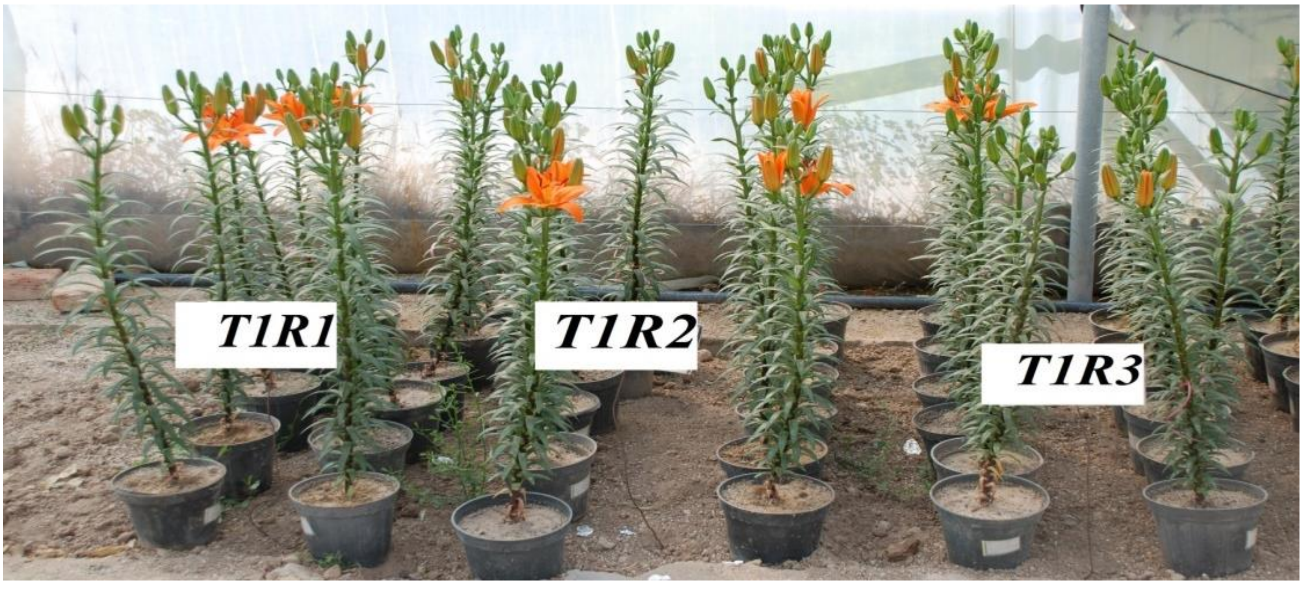

Fig. 1. Irradiated *Lilium longiflorum* cv. Tresor after cultivation.

Leaf relative water content (RWC): For measuring the plant water balance we use of the parameter called Relative water content (RWC) which is the physiological result of deficiency in cellular hydraulic situation. For evaluation the RWC two discs of leaves from average height of flowering stem were cut and weighed (fresh weight, FW) and replaced in ddH$_2$O for 2 hours to absorb the water until full turgidity in dark-place. The data from weighted discs recorded as their turgid weight (TW). The mentioned leaves were then dried at 70^{o}C for 24 h and their dry weight (DW) was registered. In consequence, RWC was recorded using the below equation (Turner, 1981):

$$RWC (\%) = [(W-DW) / (TW-DW)] \times 100$$

Electrolyte leakage (EL): For determination the amount of electrolyte leakage (EL) the discs of fresh leaves (0.5 cm diameter) were detached from the completely widen leaves from the average height of flowering stem and were replaced in closed tubes including 10 mL of ddH$_2$O and incubated at 40°C for 30 minutes then, the first electrical conductivity of the solution (EC1) was recorded using a conductometer. The leaf discs were then incubated at 100°C for 15 minutes in water bath to extrude all electrolytes, allow them to cool down to room temprature and their second electrical conductivity (EC2) was recorded again. Finally, this equation was used for calculating the electrolyte leakage (EL);

EL = (EC1/EC2) × 100 (%) (Lutts *et al*., 1996).

Leaf area and bulb diameter: Leaf area index is a dimensionless quantity that characterizes plant canopies. Leaf area meter (Li-Cor, Li-1300, USA) was used for evaluation of total leaf area. The leaves were dried at 80°C in order to determine specific leaf area (SLA). Bulb diameter also was measured by digital coulis.

Stem height: The length of plant stem was evaluated by ruler from the basal point of stem until apical meristem.

Dry and fresh weight: For measuring the plant fresh and dry weight, one of the flowers was harvested from each pot at the end of the treatment period, sterilized using deionized water and was dried fresh at 70°C for 48 h, in an oven. Then dry weight was calculated using an electronic precision balance (Sartorius, Basic, Germany).

Chlorophyll a, b and total: About 0.2 g of finely mixed of leaf tissues were extracted using 10 mL of 80% acetone. After 24 hours incubation in dark place Chlorophyll was extracted from completely blanched leaves. Then the amount of absorbance of extracted samples was recorded at 649, 665, and 470 nm by a spectrophotometer (Shimadzu UV-2550, Kyoto, Japan). For evaluating the concentration of chlorophyll, a standard method similar to Arnon, (1949) was applied and calculated as mg g^{-1} fresh weight.

Statistical design: For the experiment a completely randomized design with irradiation at 6 levels of γ-rays (0, 10, 20, 30, 40 and 50 Gy) with 3 replications for each dose and 3 bulbs for each replication were utilized. The data was analyzed with ANOVA version 9.1. The treatments mean values were contrast with Duncan's multiple range tests at $p \leq 0.05$.

Results and Discussion

Evaluation of the results to determine the appropriate irradiation dose in Lilium cv. Tresor through physiological traits: For evaluate the effect of γ-rays on vegetative alteration in plants, 9 lilium bulbs were irradiated for each dose. After completing the plant growth traits such as leaf number and area, dry and fresh weight, flowering stem length, water content of leaf relative, membrane stability index and chlorophyll a, b and total chlorophyll were evaluate. Results showed significantly difference ($p \leq 0.01$) in all measured traits except for leaf relative water content (RWC) and bulb diameter (Table 1).

Number of leaves: Results showed a significant reduction in leaf number of plants ($p \leq 0.05$) with increasing in levels of γ-rays. Control plants (without irradiation) have The most average number of leaves (86.33) whereas plants irradiated with 50 Gy gamma ray had the least (41.17) number of leaves (Table 1) as it was tremendously reduction at 40 and 50 Gy with average of 50 and 52% compare to control. Our values were in compatible with the values obtained by Ochatt *et al*., (2001) in Latyrus & Gonzales (2007) on Gladiolus. The high levels of γ-rays doses had the negative effect on the number of leaves per ornamental. It seems that alteration in plant growth regulators status inside the plant especially kinetin, can be responsible for the variation in leaf number.

Electrolyte leakage (EL): Regarding the ion leakage trait, according to the results of table 1, the difference between control and other treated plants at low doses of radiation was not significant, but there was a significant increase from 20 to 50 Gy treatments. There was a decreasing trend in membrane stability index of leaf as with increasing in irradiation dose; the membrane stability of controls was decreased from 37.11 dS m^{-1} in controls to 31.15 dS m^{-1} in irradiated bulbs at 50 Gy, which indicates the injury to the membrane and electrolytes are released from the membrane. It seems that because of cell wall collapse and reflux of ions.

Followed by gamma radiation, electrolyte leakage increased.

Leaf area: Results in Table (1) displayed the influence of gamma ray at six doses on leaf area. There was a significant decrease in leaf area between the control and different levels of treatment. The most amount of leaf area was observed in the un treated plants (60.05 cm^2), which is decreased to 37.75 cm^2 in 50 Gy irradiated samples (Table 1). Our results agree with El-Khateeb *et al*., (2016) in *Philodendron scandens*. Gamma irradiation may be effect on growth by alteration the hormonal signaling transduction pathway inside the plant cell. So the smallest leaves (37.75 cm^2) were obtained at 50 Gy dose.

Table 1. Effect of γ-rays on no. of leaf, RWC, EL and leaf area of *L. longiflorum*.

Treatment (Gy)	No. of leaves	RWC (%)	EL (ds m^{-1})	Leaf area (cm^2)
0	86.33^a	67.31^a	37.11^a	60.05^a
10	65.84^b	61.31^a	34.83^b	43.66^b
20	63.50^b	56.45^a	34.58^b	40.09^b
30	63.17^b	55.79^a	34.19^b	38.99^b
40	42.67^c	53.75^a	32.53^{bc}	38.44^b
50	41.17^c	52.57^a	31.15^c	37.75^b

In each column, means with similar letters are not significantly different using Duncan's multiple range test at 1% of probability level

Table 2. Effect of γ-rays on flowering stem height, fresh and dry weight of *L. longiflorum*.

Treatment (Gy)	Flowering stem length (cm)	Fresh weight (g)	Dry weight (g)
0	63.33^a	107.1^a	17.10^a
10	11.50^b	33.92^b	12.10^b
20	8.887^c	31.34^c	11.44^b
30	8.553^c	24.40^d	10.97^{bc}
40	8^{cd}	20.03^e	9.895^{cd}
50	6.777^d	17.03^f	9.258^d

In each column, means with similar letters are not significantly different using Duncan's multiple range test at 1% of probability level

Table 3. Effect of γ-rays on Chlorophyll a, b and total chlorophyll of *L. longiflorum*.

Treatment	Chl a (mg g^{-1})	Chl b (mg g^{-1})	Total Chl (mg g^{-1})
0	0.1407^b	0.0729^b	0.2137^b
10	0.0904^d	0.0831^b	0.1736^c
20	0.1128^c	0.0817^b	0.1945^b
30	0.1695^a	0.1281^a	0.2975^a
40	0.0845^d	0.0733^b	0.1578^c
50	0.1112^c	0.0891^b	0.2003^b

In each column, means with similar letters are not significantly different using Duncan's multiple range test at 1% of probability level

Flowering stems length: The Effect of vaious levels of gamma irr γ-rays on average flowering stem length (cm) of *Lilium longiflorum* was also shown in Table 2. Flowering stem length of plants treated with different levels of gamma ray showd significant variation as by increasing in dose of gamma ray, flowering stem length was decrease tremendously i.e., 6.77 cm at 50 Gy compare to 63.33 cm in controls which in practice, according to statistics, we saw dwarfism in the plant. The mentioned results are relying on the existence of a relationship between thicknesses of plant structure and stunting in plant which is exposure to radiation. Leaf dimension of the plants have diminished as well as increase in application dose, too. The same results were obtained by Cheng *et al*., (2010) in *Solanum tuberosum* and Aslam *et al*., (2016) in *Lilium longiflorum*. Diminish in flowering stem length can be because of the damage to growth and development aspects of plant such as cell division and cell enlargement resulted in alteration treatment. It is clear that application of high doses of gamma ray in irradiation can have a harmful effect on flowering stem length (Singh *et al*., 2000) which is one of the most effective traits in cut flowers. One of the reasons for reduction in flowering stem length seems to be related to decrease in mitotic activity of meristematic cells, auxin biosynthesis, alteration in ascorbic acid level and also disturbance in physiological and biochemical traits (Asare *et al*., 2017). From the other point of veiw, elevated doses of γ-rays chip in cell cycle detention during G2/M phase and finally growing ratio is decreased during cell division and (or) the whole genome was damaged (Preussa & Britta, 2003).

Fresh / Dry weight. The response of fresh weights of leaves and stems of *Lilium longiflorum*, to different doses of gamma rays (Table 2) revealed that interaction between controls and treated plants were significantly different. As shown in Table 2 both fresh and dry weight plant, leaves and stems of gamma irradiated plants displayed a gradual decrement as the gamma dosage increased from 10 to 50 Gy. However, fresh weight was more affected by irradiation doses than dry weight. Melki & Marouani (2010) and Uluke & Faith ozmen (2018) reported the same results in hard wheat and common bean, respectively. Also El-Khateeb *et al*., (2016) stated the same results in fresh weight of *Philodendron scandens*. Treating lilium flowers with 50 Gy dose decreased the average of plant fresh weight to minimum value (0.84%), while the average of plant dry weight irradiated with 50 Gy treatments, giving 45.9% increment over the control. Moreover, decrease in fresh and dry weights of flowering stem length could be related to the deficency in moisture content of the stem due to induced radiation stress (Abdul Majeed *et al*., 2010).

Chlorophyll a, b and total chlorophyll: Considering to the results of ANOVA a significant difference ($p \leq 0.01$) was shown among different levels of radiation. Results showed in Table 3 rely on the effect of 30 Gy dose of gamma ray in Chlorophyll a, b and total chlorophyll increasing compare to control and other treatments and then decreased slightly. When the dose of gamma ray application is high, damage to DNA structure and rate of mutagenesis are the most, but almost of the plants died or had sterility (Koornneef, 2002, Preuss & Britt, 2003).

Palamine *et al*., (2005) reported that irradiation of dracaena plant with gamma rays 20 Gray cause to diminish in chlorophylls a and b content. Also, Sakr *et al*., (2013) concluded that when *Dracaena surculosa* exposed to 10 Gray γ-ray, the plant had the most amount of chlorophyll. Exposure of maize in 10-40 kR doses of gamma radiation increased the chlorophyll contents significantly. It is suggested that variation in biosynthesis of chlorophyll in treated plants with radiation may be due to auxin synthesis (Singh, 1971). Also a literature implies that the first effect of irradiation was on the growth of meristems and the effect on auxin accumulation may be a lateral effect of the same (Giacomelli *et al*., 1967).

The effect of γ-rays on bulb diameter and leaf relative water content (RWC) of leaf were not significantly different.

Evaluation of the results to determine the appropriate irradiation dose in Lilium cv. Tresor through regression: The major part of variation studies is to recognize and identified the optimum level for the varieties. Regression in vegetative characteristics in the lilium flowers comes from gamma radiated bulbs is commonly a detector of what the flower was affected in genome. The LD_{50} dose should be recognized to determine the optimal variation level (Predieri, 2001). After irradiation by measuring leaf number and area, flower fresh weight and dry weight, flowering stem height, relative water content of leaf, ion leakage, bulb diameter, chlorophyll a, b and total chlorophyll can be regressed and estimated by algebra. The linear model evaluated the appropriate relationship between dose and the measured traits and determined the appropriate dose based on the obtained relationship. At the end of the growth time, the lilium plant was examined for the mentioned traits, then a linear model of the relationship between dose and number of leaves, leaf area, fresh and dry weight, flowering stem height, total chlorophyll was plotted. By comparing the graphs and ensuring their linearity, the desired trait was determined to the optimal dose for variation induction in lilium flower.

The linear model of the regression relationship between the amount of leaves and the amount of radiation dose and the coefficient of explanation obtained in regression analysis (Fig. 2) was equal to 0.491, ie this relationship explained about 50% of the differences between these two traits and the rest of the changes from a nonlinear relationship followed.

In the leaf area trait (Fig. 3), the obtained regression relationship explained 64% of the differences between these two traits. The regression relationship of plant fresh weight (Fig. 4) was 61%, dry weight (Fig. 5) was 78%, and flowering stem height (Fig. 6) was 50%.

The coefficient of determination of total chlorophyll was zero (Fig. 7), which means that the use of independent variables has no effect on the estimation of the regression line. Therefore, no justifiable relationship was observed between this trait and radiation dose. As a result, this trait cannot be a criterion for determining LD_{50} and is ignored.

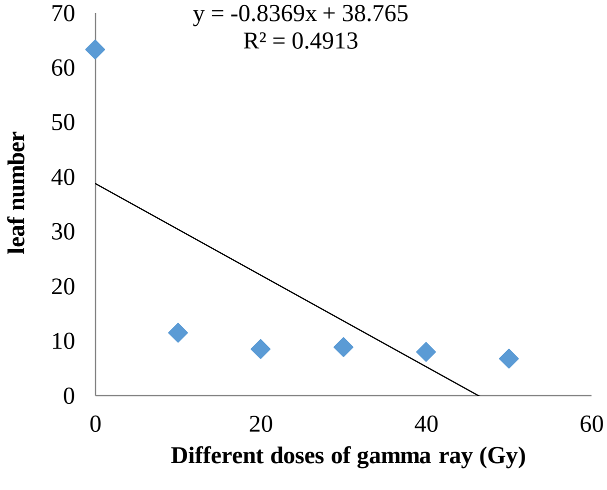

Fig. 2. Regression analysis on plants with leaf number in *Lilium longiflorum* after gamma treatments.

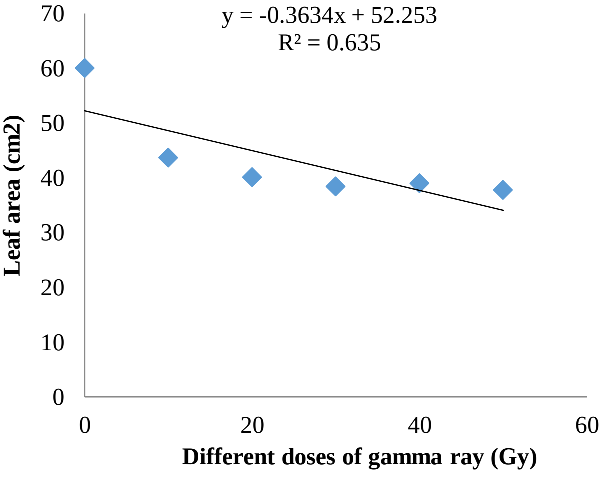

Fig. 3. Regression analysis on plants with leaf area in *Lilium longiflorum* after gamma treatments.

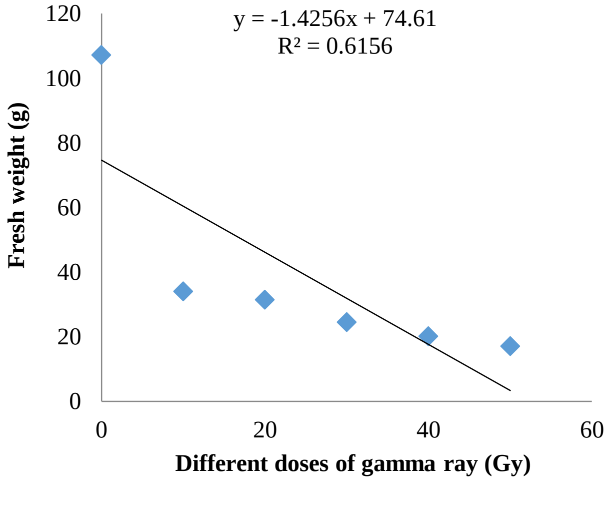

Fig. 4. Regression analysis on plants with fresh weight in *Lilium longiflorum* after gamma treatments.

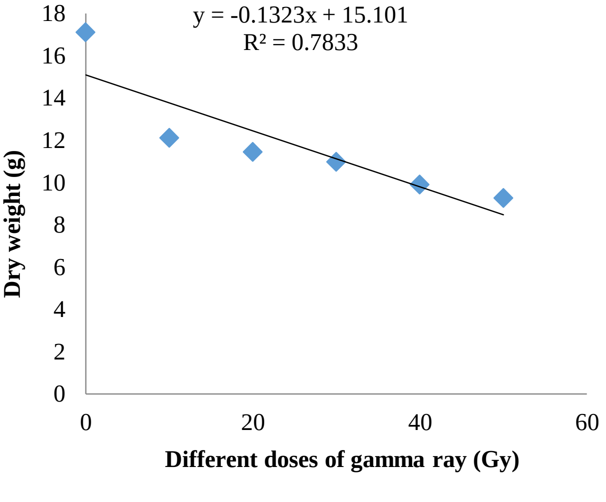

Fig. 5. Regression analysis on plants with Dry weight in *Lilium longiflorum* after gamma treatments.

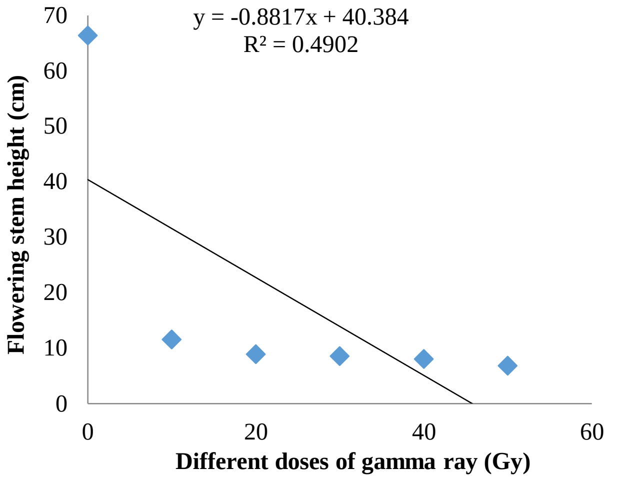

Fig. 6. Regression analysis on plants with flowering stem height in *Lilium longiflorum* after gamma treatments.

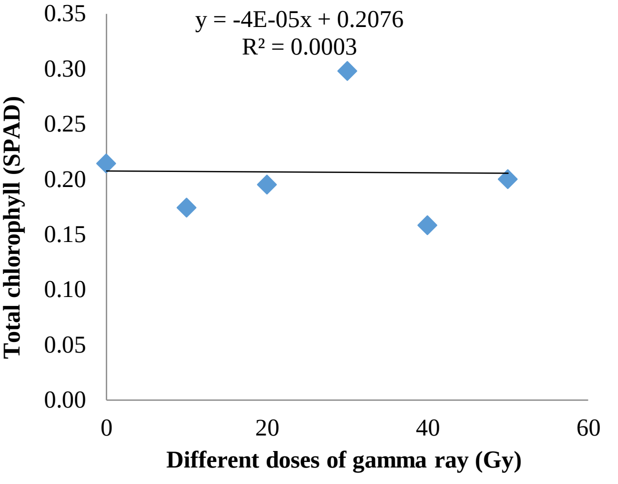

Fig. 7. Regression analysis on plants with total chlorophyll in *Lilium longiflorum* after gamma treatments.

Table 4. Explanation coefficient obtained in linear regression relation of radiation dose and number of leaves, leaf area, plant fresh weight and dry weight, flowering stem height, total chlorophyll in Lilium cv. Tresor.

Fw	Dw	Flowering stem height	Leaf number	Leaf area	Total ch
R^2= 0.615	R^2 = 0.783	R^2 = 0.490	R^2 = 0.491	R^2 = 0.635	R^2 = 0

Table 5. The dose determined in Lilium cv. Tresor based on regression equation.

Physiological traits		GR_{50}
Fresh weight	Y= - 0.425x + 74.61	-
Dry weight	Y= - 0.132x + 15.10	25
Flowering stem height	Y= - 0.881x + 40.38	-
Leaf number	Y= - 0.836x + 38.76	-
Leaf area	Y= - 0.363x + 52.25	-

Comparison of regression relationship between studied traits and radiation dose: The regression relationship of leaf number and area, flower fresh and dry weight, flowering stem height, total chlorophyll was obtained as a random variable and gamma ray dose as a constant variable. The coefficient of explanation obtained based on the linear regression relationship is shown in Table 4. Based on these results, the coefficient of determination obtained in the plant dry weight trait was higher than other traits and was a suitable criterion for determining LD_{50}.

Determination of appropriate dose (LD_{50}) in Lilium cv. Tresor: Due to the higher coefficient of determination obtained in the dry weight trait, this trait was a good indicator to determine the optimal dose for leaf number and area, plant fresh weight, flowering stem height and total chlorophyll in the samples. According to the obtained regression equation (Table 5), the desired dose can be calculated based on 50% growth reduction (Y), which in this equation x shows the dose in terms of proportion. Calculations showed that in Lilium cv. Tresor 13Gy dose will have a 50% reduction in growth in this dose. Therefore, the range of 10 to 20 Gy in this cultivar is suitable as a dose range for causing mutations. The optimal dose determined in this cultivar shows that there is a difference between different levels of radiation and even different cultivars of a plant in terms of sensitivity to gamma rays.

The effect of irradiation on vegetative traitrs and determination of LD_{50}: Gamma ray is one of the powerful agents that are able to change in physiology and biology of flowers related to the absorbed doses (Kiong *et al*., 200). Results of some projects showed that gamma rays at higher levels have a negetive effect on plants; it can be produce free radicals which are harmful effects on physiology, morphology and DNA structure of cells according to the irradiation dose.

The first step in breeding programs through mutation is to detect the optimum level for variation (LD_{50}), in which the highest amount of mutation is induced in the samples, as well as the minimum damage and losses (50% reduction in growth). Thus, it is possible to use growth factors as a measure of LD_{50} (Hines & Schmidt, 1995). In this study, irradiation of Lilium bulbs cv. Tresor with low doses in terms of growth factors was not observed compared to the control sample, but with increasing beam intensity at a dose of 50 Gy, the mortality rate increased and beyond this dose led to lack of plant growth and it was destroyed. Plant dry weight was a more appropriate criterion for determining LD_{50} in lilium, which is significantly reduced with increasing radiation dose.

Irradiation of lateral buds of Apollo Rose genotype with gamma rays showed that survival percentage, seedling height, fresh weight and also biomass of plants reduced with increasing γ-rays dose, which considering to the results of the present experiment on reducing flowering stem height and fresh and dry weight (Vedadi, 2006). It can be said that sensitivity to gamma rays and other mutagens depends on the species, cultivar, physiological conditions of plants and organs, and some environmental conditions before and after irradiation. Examination of the coefficient of determination for leaf number and area, plant fresh and dry weight, flowering stem height and total chlorophyll showed that a high percentage of changes in dry weight trait followed a linear relationship, thus a suitable criterion for determining The optimal dose was detected. The traits of leaf number, leaf area, plant fresh weight, flowering stem height were less than the linear relationship and had a lower coefficient of expression than dry weight. The coefficient of determination of zero in total chlorophyll indicates that the use of this attribute has no effect on the estimation of the regression line and is therefore not suitable for determining the desired dose. As a result, plant dry weight was found to be a very suitable criterion for determining LD_{50} for mutation. Diodi & Banerjee (2008) irradiated the root cuttings of dahlia cv. pinki with different levels of 0, 250, 500, 1000 and 1500 gamma rays. With increasing radiation dose, a decrease in viability, plant stem length, leaf number and flower size were observed in this cultivar and LD_{50} was determined in terms of survival percentage. Yu *et al*., (1977) examined different methods for determining LD_{50} and finally suggested the use of regression equation for this purpose. In this study, the optimal dose determined based on the linear regression relationship in Lilium cv. Tresor was estimated to be about 10 to 20 Gy. In a similar study, woody buds of seven cultivars of rose with five different colors were exposed to various levels of γ-rays (0, 3, 4 and 5 rad). The results showed that in response to radiation, some growth factors such as number and length of branches, survival rate, flower number, flower weight and pollen productivity decreased. The optimum dose for white cultivars was low in comparison with other cultivars, and these cultivars were suitable for inducing mutations. Phenotypic changes in leaves, flowers, and growth habit were observed (Datta & Datta, 1998). Stem cuttings of *Hibiscus sinensis* were exposed to various levels of 10, 20, 30, 50, 70, and 100 Gy γ-rays to cause mutations. Severe growth was seen in the buds (Srivastava & Mishra, 2005). The effects of various levels (100, 150, 200, 250, 300, 350, 400, 450 and 500 Gy) of γ-rays (60Co) on vegetative traits of plant and survival rates of the seedling were studied in common bean (*Faseolus vulgaris* L.). It was suggested that low dosage of γ-rays effect on seedling fresh and dry weight in both root and shoot. Also the correlation was observed between vegetative characteristics of plant and stem thickness was negative, and the stem diameters are thickened as well as increase in γ-rays (Ulukapi & Ozmen, 2018). The LD_{50} In two cut rose cultivars 'Mourossia' and 'Apollo' calculated as survival rate at 67 and 66 Gy, respectively. Considering to the other measurd traits, the doses of 70 and 68 Gy cause to 50% reduction in flowering stem length in 'Apollo' and 'Maroussia' respectively. Thus it is concluded that the best dose of gamma radiation for the mentioned varieties can be determined between 60 to 70 Gy (Kahrizi *et al*., 2011). The most effective dose of gamma radiation for induced mutagenesis in African violets (*Saintpaulia ionantha* H. Wendl.) was determined at 15 Gy (Seneviratne & Wijesundara, 2007).

Conclusion

According to the results of LD_{50} determination, it was found that there was a difference in gamma ray sensitivity between the studied traits for causing mutations in Lilium cv. Tresor by examining different indices (leaf number and area, plant fresh weight and dry weight, flowering stem height, total chlorophyll) after gamma ray irradiation based on higher coefficient of explanation in relation to linear regression. A suitable criterion for this purpose in plant was dry weight. The results showed that the modification of lilium through mutation using gamma radiation can be considered as a suitable breeding method. Because in a short time and with the least chromosomal damage caused by gamma radiation, good results can be achieved in modification of lilies.

This experiment was performed to identify the optimum dosage of acute gamma radiation for bulbous plant. Results from this preliminary study are useful for subsequent work especially in bulbous plant. *In vivo* irradiated plants showed a significant variation in different vegetative characteristics compare to controls. Based on the 50% plant survived after 11^{th} week, the optimum dose is estimated between 25.5 and 27.5 G-ray. Rely on the results, with increasing in dose of gamma radiation, decrease in flowering stem length and prevention of root production were obtained. These observations are usable in designing a breeding procedures depend on variation changes in lilium cv. tresor cut flowers.

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