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Effect of gamma radiation on morphological changes and vegetative growth in sunflower (*Helianthus annuus* L.)

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Abstract

Changes in the genetic structure through gamma radiation of the plant can produce physiologically and bio-chemically efficient plant type with respect to increase production and attractiveness. For mutation breeding, scientists use gamma radiation to produce effective plant types. Beforehand, an effective range of radiation should be identified for possible suitable mutational effect. In this context, the present study was conducted to determine the effective doses of gamma radiation for mutational breeding of sunflower (H. annuus L.) on vegetative growth performances. Sunflower seeds were exposed to different doses of gamma radiation (0 Gy, 100 Gy, 200 Gy, 300 Gy, 400 Gy and 500 Gy) as treatments. The experiment was laid out in the Complete Randomized Design (CRD) with six treatments in four replications. Each replication comprised ten plants in field. Treating the sunflower seeds with gamma radiation has a significant ($p<0.05$) effect on the tested parameters except on germination percentage. Untreated plants with gamma radiation and plants treated with lower doses of gamma radiation (100 and 200 Gy) showed comparable values in percentage survival as 100%, 94.5% and 92.5%, respectively. LD50 value was recorded as 440 Gy. In the first two weeks of growth, untreated plants and plants treated with 100 Gy with gamma radiation showed similar values in a number of leaves and plant height. Later in the 4th and 6th week highest values were found where plants were not exposed to gamma radiation. A reducing trend in values in the measured variables has been observed with the increase in gamma radiation doses. The lowest values in all observations were recorded by the seeds exposed to 500 Gy, which is the highest dosage used in this experiment. The plants treated with the dosages higher than 100 Gy exhibited misshapen, deformed and abnormalities in leaves compared to the control, but the changes were not persisted. Hence, it could be suggested that exposing the sunflower seeds to gamma radiation dose has caused the negative effects on vegetative growth performances. Further, exposing sunflower seeds close to 440 Gy may have the potential to produce the sunflower variations especially dwarf plant stature with desired characteristics and optimum survival for domestication in future studies.

Keywords: Gamma radiation, Growth parameters, Morphological characters, Mutation, Sunflower

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INTRODUCTION

Sunflower (*Helianthus annuus* L.) is an oleaginous crop belonging to family Asteraceae. It contains 2n = 34 numbers of chromosome. It is the world's fourth largest oil-seed crop. Sunflower seeds are used as food and its dried stalk as fuel. It is also used as an ornamental plant and was used in prehistoric celebrations in ancient time (Muller *et al.,* 2011). Sunflower plants can be grown successfully in a healthy environment with rich, fertile soil. The flower head has two types of floret; ray florets are on outside whorls and surround the disc florets in the center. The floret arrangement attracts bees and butterflies (Blackman *et al.,* 2011). Due to its short wavelength, gamma radiation has a high penetrating power and is an example of ionizing radiation. This is released by some of the most popular breeding programs, such radio induced mutagenesis, to create genetic diversity that hardly occurs in nature (Iglesias-Andreu *et al*., 2010). One way of creating genetic variation in sunflower is by inducing mutations through gamma radiation.

In order to improve plant production and traits, mutation breeding, the most beneficial and crucial technology for sunflowers (Kumar & Ratnam, 2010), is successfully applied in sunflower breeding (Gvozdenovic *et al*., 2009). Mutants with altered, better vegetative and oil-related features in sunflower have been produced using a variety of mutagens, including heat, fast neutrons, X-rays, beta rays, gamma rays, ultraviolet, and infrared radiation. These mutants can be cultivated (Cvejic, 2009). In most cases, radiation-induced changes can cause undesirable effects and variation in the phenotype, whereas in some cases, these mutations may be of interest to the breeder. Radiation-induced useful variation can bring about positive changes in yield, resistance to biotic and abiotic stress factors, new floral traits of ornamental value, etc. (Gómez *et al.*, 2017). Radiation technology is broadly used to generate a greater number of mutations, which supports to introduce novel, improved variants compared to the existing types. Although sunflower is mainly grown as an oil crop, there is an aesthetic value too. It is naturally grown as a tall and straight single statured plant in natural habitats with yellow inflorescence. Therefore, it is worth introducing as a plant embedded with dwarfness and different floral colours to be introduced as an attractive ornamental plant in favour of domesticated indoor plant gardening. However, with natural selection and conventional breeding, it is difficult to obtain desired variations in the plant. Therefore, exposing seeds of this particular plant for inducing mutations through gamma radiation is important. Thereby, as the first step in a sequence of experiments planned for inducing mutations, identification of the lethal dose (LD50 value) followed by an effective dosage is of prime importance. Accordingly, the present investigation was conducted with the objective of identifying the effects of gamma radiation on the morphology and vegetative growth of sunflower.

MATERIALS AND METHODS

Experimental location

This experiment was conducted at the Institute for Agro-technology and Rural Sciences, Weligatta, Hambantota, which is situated in the dry zone of Sri Lanka, during the period of January to March 2020. Annual average rainfall of the area is 1063mm. Reddish Brown Earth is the most common type of soil found in the study area.

Planting material

Sunflower seeds from Onesh Agri Pvt Ltd, Kent Road, Colombo, Sri Lanka were used for the radiation treatments. The seeds were tested for their germination prior to expose to gamma radiation by placing 20 seeds in a moistened paper tissue inside a petri dish.

Experimental design and radiation treatments

Seed samples were treated with different doses of gamma radiation from a Cobalt 60 gamma source available at Horticultural Research and Development Institute (HORDI) at Gannoruwa, Sri Lanka. The treatments consisted of 0 Gy, 100 Gy, 200 Gy, 300 Gy, 400 Gy and 500 Gy doses of gamma radiation. Each treatment contained 40 seeds. The treated seeds were directly planted in pots containing 1:1 mixture v/v of sand and compost. When the seedling produced four true leaves, they were transferred to the field with recommended spacing about 60cm × 25cm and following all cultural measures recommended for *H. annuus* by the Department of Agriculture, Sri Lanka. The experimental design was a Completely Randomized Design with four replications. Each replication contained 10 plants and altogether, 40 experimental units were used for each treatment. Germination percentage at 7 days after seeding, morphological changes in plants, percentage survival, plant height and number of leaves were recorded during the plant growth of the treated plants.

Germination percentage

Number of seeds germinated was counted until two weeks and the germination percentage was calculated as follows:

$$\text{Germination \%} = \frac{No.\ of\ seeds\ germinated}{No.\ of\ seeds\ planted} \times 100$$

Percentage survival

Survival rate of the plants was calculated two weeks after transferring to field. The function used is:

$$\text{Survival \%} = \frac{No.\ of\ plants\ survived}{No.\ of\ seeds\ germinated} \times 100$$

Plant height at 2nd, 4th and 6th week

Plant height was measured at 2nd, 4th and 6th weeks after planting of the seedlings and height was measured in cm from the soil surface to the apex of the topmost leaf.

Number of leaves at 2nd, 4th and 6th week

Number of leaves was measured at 2nd, 4th and 6th weeks after planting in pots manually by counting.

Statistical analysis

The obtained data were statistically evaluated using ANOVA in the SAS 9.1.3 statistical program, and Duncan's Multiple Range Test (DMRT) was used to examine the differences between treatment means at the 0.05 significant level.

RESULTS AND DISCUSSION

Morphological changes

The plants treated with the dosages higher than 100 Gy exhibited misshapen and deformed leaves compared to the control. This nature was recovered with leaf maturity and perhaps this misshapen nature could be brought about by the physiological stress caused by the strong radiations (Plate 1) Also, the plants exposed to all radiation doses beyond 100 Gy showed abnormalities in leaves (Plate 2) during the early leaf developmental stages. They showed white mottles on leaf blades but with maturity, the mottles disappeared and leaves visualized a normal appearance. The changes in these developing leaves may be due to the physiological changes caused within the cells by radiation stress. They might have developed green pigments to mitigate the radiation effects with their maturity. No any variations and deformities in the flower colour and structure were observed.

A mutagenesis study on *Calendula officinalis* belonging to the family Asteraceae by Tiwari and Kumar (2011) indicated that exposing the seeds of *C. officinalis* to gamma radiation dose ranging from 2.5 Krad – 7.5 Krad showed morphological abnormalities in leaf and plants. Further increase in radiation dose increased the abnormal leaves. Similar results were observed by Kapoor *et al.* (2014) in *Glebionis segetum*. They mentioned that the percentage of abnormal leaves significantly increased with the increase in gamma radiation dose. Further, maximum deformed plants were recorded in plants treated with 100 Gy. This significant development of abnormalities may be due to radiation damage of the irradiated plants, specifically chromosomal breakage (Gaul, 1970), which generally causes physiological, morphological and cytological disturbance by gamma radiation. Same The same trend was recorded in Chrysanthemum by Misra *et al.* (2009) that, per cent abnormal leaves significantly increased with the increase in gamma rays dose over the control. The different types of leaf abnormalities included change in leaf shape and size, margins, apex, fission and fusion were recorded after irradiation.

Plate 1: Sunflower seedlings at 2 weeks after seeding: a) Control plant; b, c, d) plants treated with gamma radiation above 100 Gy


Plate 2: Sunflower plants exposed to gamma radiation showing mottled leaves

Germination percentage

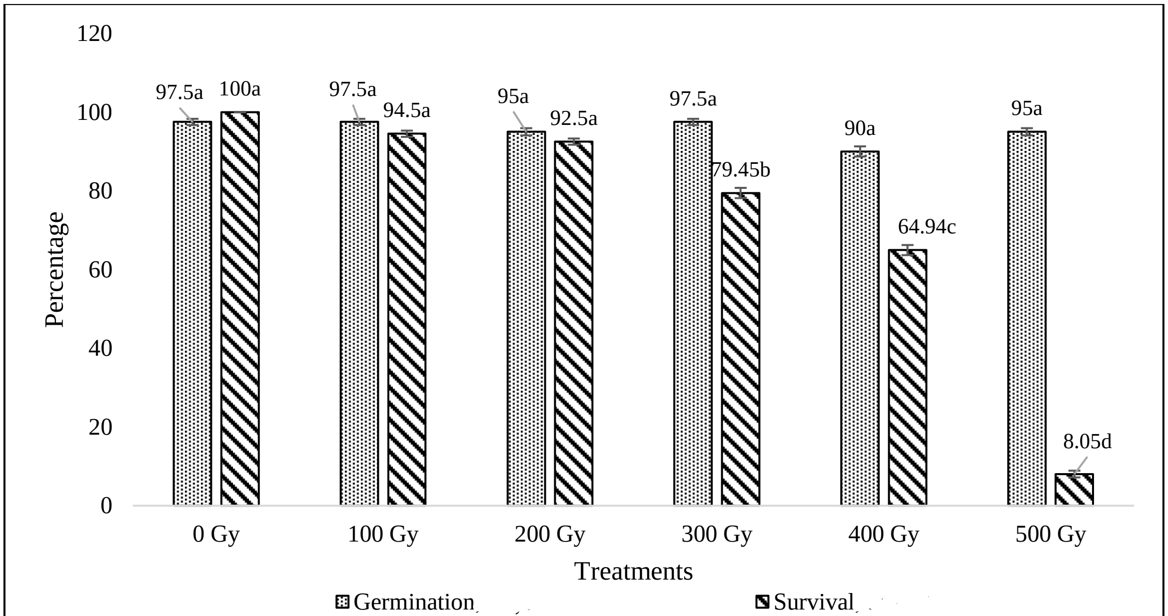
Early germination test of the seeds prior to gamma radiation treatments showed 100% of germination. Treating the sunflower seeds with different doses of gamma radiation did not ($p>0.05$) affect seed germination significantly (Plate 3). With increase in dosage of gamma radiation, they produced weak and slender seedlings (3b) compared to untreated seeds (Plate 3a). As indicated by Rifnas *et al.* (2019), increasing trend in gamma radiation dose decreased the germination percentage of sunflower seeds. Further, as mentioned by Diaz *et al.* (2018), when exposing sunflower seeds to different doses of gamma radiation, the highest percentages in seed germination occurred in the control treatment and with doses of 100 and 200 Gy, those values were 95% and 93% respectively. Further, increase in gamma radiation dose caused a progressive decrease in seed germination. A study on marigold by Latha and Dharmatti (2018) too have proved that seed germination decreased with increase in the dose gamma radiation.

Plate 3: a) Untreated sunflower plants at 2 weeks after planting, b) Sunflower seeds treated with 500 Gy dose at 2 weeks after seeding

Percentage survival

There were significant ($p<0.05$) differences between the treatments on the survival rate of sunflower plants (Figure 1). Plants not exposed to gamma radiation and plants treated with 100 Gy and 200 Gy reported similar values in the percentage survival of plants. The survival rate of plants decreased with increasing doses of radiation and the least survival was observed in the highest dose of 500 Gy. The Figure 2 clearly illustrates that the 50% survival rate (LD 50) was observed at 440 Gy of gamma radiation. These results could possibly be due to the negative impacts of radiation on tissues that might cause damage at the cell level, including physical or chromosomal level or physical and chromosomal as combined.

Similar results were obtained in marigold by Singh *et al.* (2009) that control plants exhibited 100% survival rate with normal growth and survival rate decreased with increase in radiation dose. Highest mortality was recorded with 400 Gy and the LD 50 value was determined above 400 Gy of gamma radiation. The sunflower is a susceptible species to high amounts of radiation, and a study by Diaz *et al*. (2018) showed that as the radiation dose increased from 200 to 900 Gy, the percentage of survival declined. As a result, the application of 500 Gy reduces survival by 54.9%. As indicated by Latha and Dharnatti (2018), when marigold seeds were exposed to gamma irradiation, the treatment with 500 and 600 Gy showed less survival and higher lethality. Moreover, several studies (Giriraj *et al.*, 1990; Kumar and Ratnam, 2010) on sunflower have indicated that the greater doses of gamma-rays were noted and observed as harmful and fatal to sunflower crop and, consequently, the lesser doses were considered as significant and valuable. The survival of seedlings was reduced with the increased dose of mutagen in sunflower varieties.



Values reflect the mean and standard error. According to DMRT, means with the identical superscripts do not differ significantly at the 0.05 level of probability.

Figure 1: Effects of different doses of gamma radiation on germination percentage and survival rate of sunflower (*Helianthus annuus*)

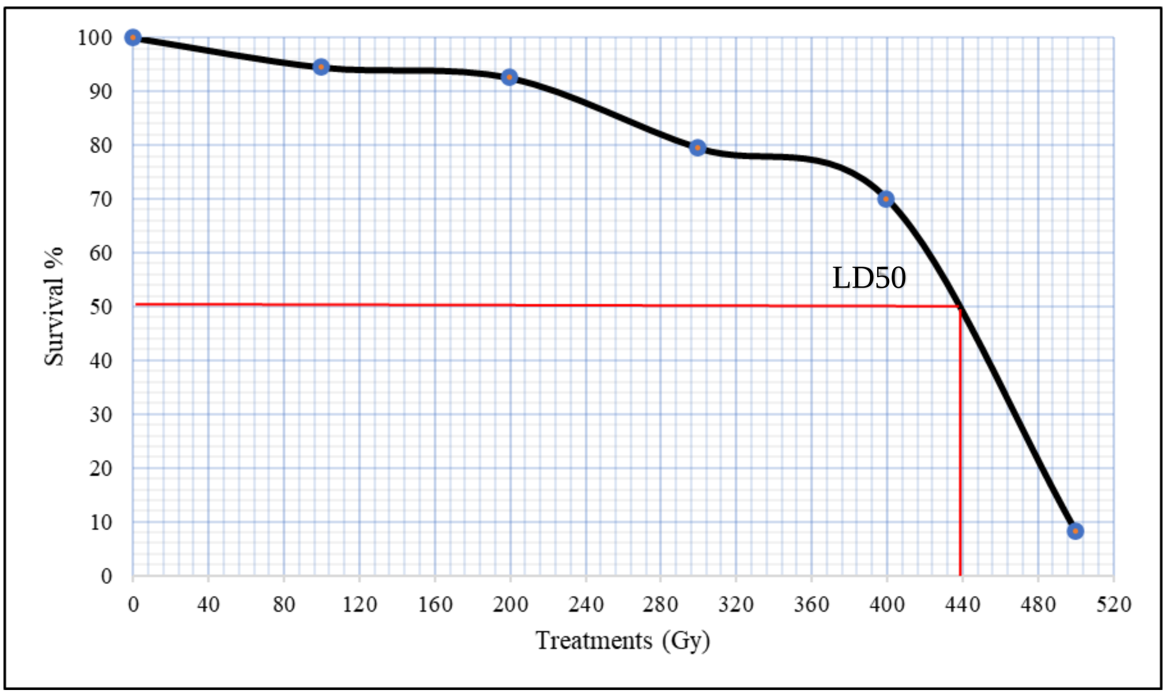


Figure 2: LD 50 value of gamma radiation dosage for sunflower

Number of leaves

It was found that there were significant ($p<0.05$) differences between the treatments on number of leaves at 2nd, 4th and 6th week of planting (Table 1). The number of leaves was significantly reduced with the increase in gamma radiation dose. Lower dose of gamma radiation (100 Gy) did not significantly affect the plant height at 2nd week. The highest values on the number of leaves at 4th and 6th week were obtained when plants were not treated with gamma radiation. The lowest leaf number at 2nd, 4th and 6th weeks were recorded by the plants treated with 500 Gy.

A decrease in auxin supply, reduced cell division, respiration and physiological disturbances may cause a reduction in the number of leaves per plant. Similar results have been reported in marigold with a drastic reduction in leaf number at 600 Gy (Latha & Dharmatti, 2018).

Table 1: Effects of different doses of gamma radiation on number of leaves per plant

Treatment	Number of leaves		
	2nd week	**4th week**	**6th week**
T1 – 0 Gy	5.75±0.06^{a}	14.03±0.07^{a}	18.90±0.28^{a}
T2 – 100 Gy	4.68±0.13^{ab}	12.40±0.19^{b}	16.60±0.13^{b}
T3 – 200 Gy	4.53±0.07^{b}	12.28±0.15^{bc}	15.93±0.11^{b}
T4 – 300 Gy	3.90±0.07^{b}	11.10±0.09^{bc}	15.68±0.05^{b}
T5 – 400 Gy	3.90±0.16^{b}	10.98±0.12^{c}	15.20±0.21^{b}
T6 – 500 Gy	0.60±0.07^{c}	8.75±0.15^{d}	12.50±0.40^{c}
Sig	*	*	*

Values reflect the mean and standard error. According to DMRT, means with the identical superscripts do not differ significantly at the 0.05 level of probability. At p=0.05, "*" denotes significant and "ns", not significant.

Table 2: Effects of different doses of gamma radiation on plant height of sunflower

Treatment	Plant height (cm)		
	2nd week	**4th week**	**6th week**
T1 – 0 Gy	7.88±0.10^{a}	16.22±0.17^{a}	41.55±0.41^{a}
T2 – 100 Gy	6.99±0.10^{ab}	13.74±0.15^{b}	35.45±0.32^{b}
T3 – 200 Gy	6.10±0.13^{b}	13.26±0.09^{b}	33.28±0.50^{b}
T4 – 300 Gy	4.27±0.12^{c}	10.05±0.022^{c}	30.70±0.85^{bc}
T5 – 400 Gy	3.49±0.11^{c}	9.75±0.06^{c}	25.33±0.59^{cd}
T6 – 500 Gy	1.87±0.10^{d}	7.53±0.24^{d}	21.75±0.37^{d}
Sig	*	*	*

Values reflect the mean and standard error. According to DMRT, means with the identical superscripts do not differ significantly at the 0.05 level of probability. At p=0.05, "*" denotes significant and "ns", not significant.

Plant height

The different doses of gamma radiation have significantly affected the plant height at 2nd, 4th and 6th week of planting (Table 2). Untreated and plants treated 100 Gy showed similar values in plant height at the 2nd week however, in the 4th and 6th week the highest value was recorded by the untreated plants (Plate 4a). The lowest value was found in plants exposed to the highest gamma radiation (Plate 4b) indicating a negative impact of radiation on plant height. Reduced growth of radiated plants may be due to the disturbances in physiological processes, reduction in auxin supply and reduced rate of cell division and respiration.

Latha and Dharmatti (2018) reported similar results in marigold where exposing the marigold plants to higher doses of gamma radiation caused a reduction in plant height significantly.

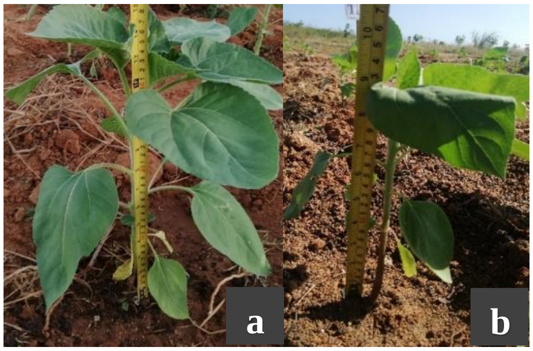


Plate 4: a) Untreated sunflower plants at 6 weeks after planting, b) Sunflower plants treated with 500 Gy dose at 6 weeks after planting

Minimum plant height was recorded in plants treated with 600 Gy dose of gamma radiation. In chrysanthemum (Bajpay and Dwivedi, 2019) and African marigold (Singh *et al.*, 2009) have also been reported comparable observations. However, Diaz *et al*. (2018) have reported that contrasting observations in sunflower. According to their study, although plant height had decreased at higher doses of gamma irradiation, the lower doses of 100 Gy and 200 Gy had a stimulating effect on plant height.

CONCLUSIONS

The results have revealed that there is no notable effect of radiation doses up to 500 Gy on germination of sunflower seeds. The increased doses of radiation induce morphological changes and pigment development; however, these changes do not become persistent and automatically recover with maturity implying the incidences of temporary stresses. The plant growth in terms of survival rate, plant height and leaf number showed a negative trend with increasing dosages of radiation. Therefore, it could be concluded that it is possible to develop a dwarf plant stature with a smaller number of leaves using appropriate gamma radiation dosage as with low gamma radiation. It needs further investigations to identify the most appropriate strength of gamma radiation to induce desired characteristics in sunflower plant such as total biomass yield, flower quality characteristics and yield of seeds and other important economic characters.

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