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Effect of Gamma Radiation on different Soybean Varieties (*Glycine max* L. Merrill) in M_1 Generation

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Abstract: This In this study, upon the application of gamma radiation in 0, 50, 100, 150, 200, 250, 300, 400 and 500 Gy doses on defiance, general and iraquous soybean varieties; determining GR_{50} doses with their effects on the germination percentage, seedling height of the M_1 generation of soybean varieties that have been grown in greenhouse environment, has been aimed. The averages that have been determined for gamma doses in terms of the traits that have been examined on the soybean varieties have been compared statistically via analysis of variance and regression.At the end of the research, it has been found out that soybean varieties are effected in a negative way depending on increasing gamma doses, and that the gamma dose that is to be used in order to obtain new varieties and create variation in soybean differs according to the varieties, however, the GR_{50} doses that can be used without causing any decrease in seed viability are 229 Gy for defiance, 248 Gy for general and 265 Gy for iraquous.

Keywords: Soybean, radiation, GR_{50} dose, seedling height, germination.

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1. Introduction

Soybean is today known as an agricultural product that has a high economical value worldwide (Xing et al., 2018; Xu et al., 2018; Zhong et al., 2016). Soybean is becoming increasingly important in agricultural fields today. The development and breeding of soybean is becoming increasingly important in agricultural fields today (Sleper and Shannon, 2003).

Plant breeding is making use of plants in terms of heredity, in other words, it is developing plants in the desired direction by utilizing existing genetic variation (Chaudhary et al., 2019).The aim in plant breeding works is to find and obtain varieties, which have high productivity and quality and are suitable for the climatic and soil conditions of large areas, or to improve the inadequate aspects of the varieties in hand (Çelik and Atak, 2017; Majeed et al ,2018).

Plant breeders utilize the potential of wild species and advance techniques for improvement including natural mutation, by extension, the utility's being limited. It is important that agriculturally feature traits in plants are generated via artificial mutagenesis (Khan,2019; Kumawat et al., 2019). Different mutagens can be used in mutation breeding depending upon the plant species, environment ,and method of application. In mutation breeding, obtaining the highest mutation frequency with the least damage is aimed (Beyaz and Yildiz, 2017; Prasanna and Jain, 2017).

Many researchers have focused on the use of physical and chemical mutagens in expanding variability of plants. Mutant individuals have been developed in many plants using gamma radiation studies that have been carried out to generate genetic variability. Some of these studies include improved individuals that possess important agronomical traits such as rust resistance in oat (Konzak,1954), flower color in chrysanthemum (Susila et al., 2019), earliness (Manjaya and Nandanwar, 2007), seed color (Karthika and Subba, 2006) and diseases resistance (Tulmannneto et al., 1988) in soybean and unshelled rice(Singh,2006), semi-dwarfism in rice (Ramchander et al., 2018), disease resistance(Tran et al., 2017), salinity (Bagheri et al., 2018) and resistance against cold, grain quality and resistance against tilting in unshelled rice (Sharma and

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Singh,2013; Tabasum et al.,2011), color of flower in rose (Datta ,1987), oil, protein and productivity quality in sesame (Hassan et al.,2018), heat resistance in wheat (Hong et al., 2018).

The determination of the most suitable dose of mutagen is very important in mutagenesis. It is well-known that the harmful effects of radiation change depending on the plants that are used and types of mutagens,and increase as the dose of the mutagen increases. Physical and chemical mutagens usually generate physical damages, gene mutations, and chromosome mutations (Çelik and Atak, 2017; Ibrahim et al., 2018).

The physiological damage caused by radiation appears in M_1 generation. However, it is not passed on to other generations. Among the physiological defects that are seen in plants; damages like delayed germination, delay or decrease in the plant's appearance, regression in growing, chlorophyll deficiencies, decreased fertility and decreased survival rate can be counted. Physiological damage can appear due to the chromosomal or extrachromosomal origin and an increased rate of radiation also increases the amount of physiological damage. The reasons in physiological damaging can have chromosomal or extrachromosomal origins (Beyaz and Yildiz, 2017; Çelik and Atak, 2017). In the development of plants, the purpose shouldn't be affected by genetic factors That is, it is expected that as little physiological damage as possible, but at the same time a high degree of variation.

As the dose increases,both physiological damage and a high degree of variation also increase, and as a result, the plant cannot survive. In high doses of radiation, even if a plant survives, it cannot reproduce. Therefore dose limits should be determined carefully, and for that, the concept of GR_{50} has been widely recommended (Conger and Konzak, 1977). This value is the mutagen dose that generally reduces seedling height by 50% compared to control; it is different for each plant species, and it should be determined before starting the mutation breeding program. Lower limits of the mean growth reduction ($GR_{50)}$ are recommended as optimum dose limits in plant improvement studies. GR_{30} dose value corresponds to the useful dose limits that are used for this purpose (Conger and Konzak, 1977; van-Harten ,1998).

In mutagenic treatments for plants, there is a correlation between M_1 mutation frequency and physiological effects such as seedling hight there and survival in M_1 generation . Therefore determining the seedling damage in M_1 is important in mutation breeding ,As a matter of fact, researchers have taken seedling height as a criterion in the mutagenesis experiments (Çelik and Atak, 2017).

Generally, various parts of plants have different levels of sensitivity to radiation, and plants' parts that are to be irradiated change depending on the purpose. The material that is preferred the most in irradiating is seeds (Borzouei et al., 2010). The reason for this is that seeds can be irradiated several physical conditions, seed moisture can be adjusted to the desired level, mutants being able to be obtained in numbers and irradiation environment's being able to be changed (Briggs and Konzak, 1977).

This research has been conducted to determine the effects of different doses of gamma radiation, which was applied on the seeds of three different soybean varieties, and various GR_{50} doses.

2. Materials and Methods

In this study, moisture ratios of the defiance, general and iraquous soybean varieties, which were supplied from the Black Sea Agricultural Research Institute. To determine the moisture content, ground soybean seed samples were stored in the oven at 130 °C for 1 h. Moisture content was determined by using the first and last weights of ground soybean samples (Conger et al., 1966).

Seed moisture contents were 8.3% (defiance), 10% (general) and 6.6% (iraquous). As for the seeds, approximately 900 seeds (for every dose and control group) were put in polyethylene sacks. Seeds were prepared and exposed to 0, 50, 100, 150, 200, 250, 300, 400 and 500 Gray (Gy) of gamma radiation dose groups using Cesium- 137 (Cs^{137}) source. The doses were applied on the same day and as single doses. Subsequently, the irradiated seeds were preserved in a refrigerator at 4 °C until sowing-time.The irradiated seeds were planted into plastic boxes, which were in 27 × 41 × 8 cm in size and had soil (pH= 5.7- 6) that consisted of a mixture of 30 % turf, 30 % decomposed leaves, 30 % pine needles and 10 % perlite.The experiments were layout at the randomized complete blocks with three replication. Since the appearance of the seeds of three different soybean varieties, their germination numbers were counted every day for 10 days, and germination percentages were determined. The effect of radiation was studied on the 14^{th} day when development ceased on the first true leaves of the soybean seedlings. M_1 seedling heights from the surface of the soil were determined to determine the GR_{50} doses of the seedlings in each treatment.

Statistical evaluations of the data, which were obtained at the end of M_1 greenhouse experiments, were done using analysis of variance and regression analysis according to SPSS 10 computer program.

3. Results

In this study, the effects of defiance, general and iraquous soybean varieties, which were irradiated with 0, 50, 100, 150, 200, 250, 300, 400 and 500 Gy on germination and seedling height have been studied separately. The moisture ratio of defiance, general and iraguous varieties were 8.3, 10 and 6.6 %, respectively. Significant negative correlations (R^2 = 0.92; R^2 = 0.92; R^2 = 0.88) have been observed between the radiation doses that were applied on gamma radiation and germination percentages of Defiance, General and Iraquous varieties. A decrease associated with increasing doses of gamma radiation compared to control on the three soybean varietieshas been detected in the germination of the seeds (Fig. 1).

Spot chlorosis has been observed on the first true leaves of the M_1 seedlings of the three different soybean varieties. The doses in which spot chlorosis was particularly apparent were 200, 250, 300, 400 Gy(Fig. 2). Since plant development above 400Gy only stay at cotyledon level, spots could not be observed. To determine the effect of gamma radiation on M_1 seedling length of irradiated, and control seedlings were measured (Table 1).

Average seedling heights belonging to the control group of the soybean plant have been measured as 25.29±1.973 cm for Defiance variety with 8.3 % moisture ratio; 14.94±5.602 cm for General variety with 10 % moisture ratio; and 19.05±4.211 cm for Iraquous variety with 6.6 % moisture ratio. It has been determined that there were decreases in the seedling heights compared to control. That is, in parallel with the increase of the dose of radiation was increased damage.

GR_{50} dose for Defiance soybean variety has been determined as 229 Gy, GR_{50} dose for General has been determined as 248 Gy, GR_{50} dose for Iraquous has been determined as 265 Gy; and the doses of gamma radiation, which would be applied on the seeds on the soybean varieties, have been determined as 200 Gy and 250 GyThe relationship between seedling heights of Defiance, General and Iraquous soybean varieties and gamma radiation has been demonstrated in Fig. 3 drawing a regression curve.

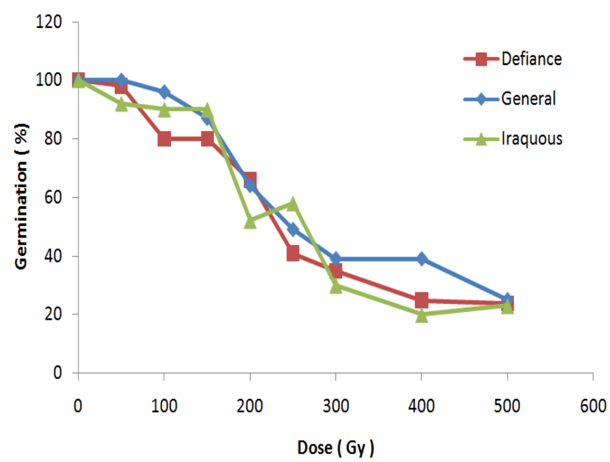

Fig.1. Effect of exposure to gamma rays on seed germination of three different varieties.

Table 1. The effect of gamma radiation on the germination percentage and seedling height of the 14th day in defiance, general and iraquous soybean varieties (± Standard deviation).

Dose (Gy)	Defiance		General		Iraquous	
	Germination (%)	Seedling Height (cm)	Germination (%)	Seedling Height (cm)	Germination (%)	Seedling Height (cm)
0	100	25.29±1.97	100	14.94±5.60	100	19.05±4.21
50	98	24.86±1.81	100	16.89±1.63	92	17.56±4.59
100	80	20.24±2.37	96	14.35±7.51	90	17.28±1.67
150	80	20.28±1.39	87	13.03±2.11	90	17.13±3.68
200	66	16.67±1.53	64	9.51±2.49	52	9.98±0.85
250	41	10.44±0.23	49	7.33±1.39	58	11.13±3.07
300	35	8.89±1.31	39	5.88±1.47	30	5.75±1.61
400	25	6.24±0.56	39	5.79±1.12	20	3.74±2.26
500	24	5.98±0.32	25	3.67±0.85	23	4.45±0.99

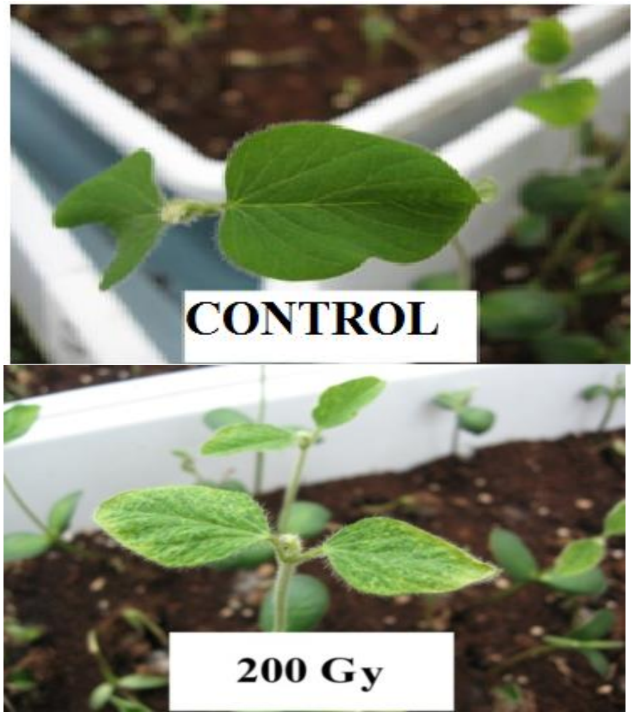

Fig .2. The first true leaves on the seedlings, which were grown out of the seeds that had been irradiated at 200 Gy with control, and spot chlorosis in iraquous soybean variety.

It has been determined that seedling height is affected by increased doses of radiation in all the three soybean varieties and that this relationship is significant ($p<0.05$).

4. Discussion

In this study, the effects of radiation on soybean plant which is of great economic and agricultural importance and its response to radiation were investigated. In mutation breeding experiments, seeds are the most suitable material to be irradiated with gamma radiation. A moisture ratio of seed is an important factor that changes the mutagenic effect of radiation. Because there is a close relationship between the effect of radiation and moisture ratio of seed, and decrease of moisture ratio in seed increases sensitivity to radiation (Bado et al., 2015; Oladosu et al., 2016). However, sensitivities of the seeds of various plants to radiation are also different from each other at the same moisture ratio (Hase et al., 2018). Because plants differ in their responses to radiation due to their characters peculiar to the family that they belong to. Doses of radiation applied on various plants also differ, by extension (Deshmukh and Malode, 2018; Gowthami et al., 2017; Jan et al., 2012).

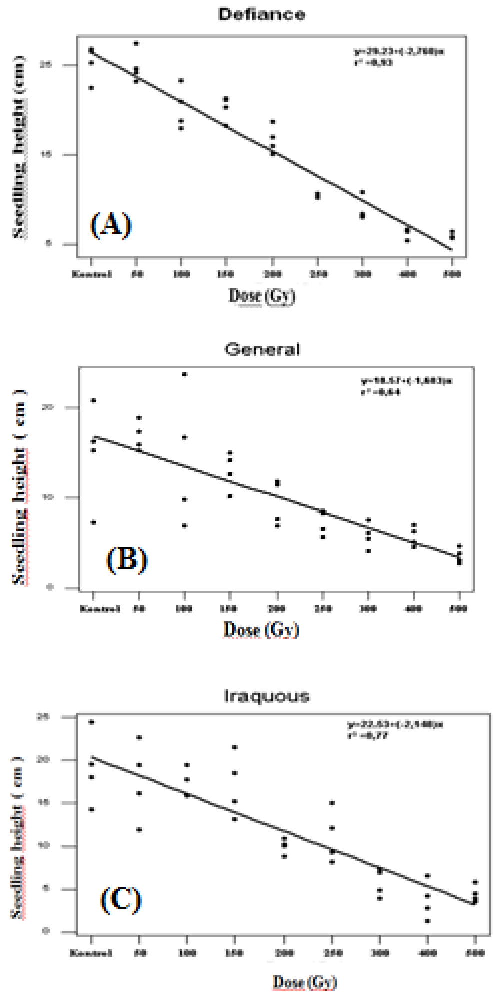

Fig.3. Relationship between seedling heights of defiance, general and iraquous soybean varieties and gamma radiation

In this radiobiological study, which has been done as a pilot study; no effect of low gamma radiation doses on the seeds of the three different varieties of soybean. The germination percentages in each three soybean varieties have been observed reduction depending increased doses of radiation. The activity level of radiation in a living being increases with the existence of oxygen at the time of irradiation (Ozalpan, 2001). Damage of gamma radiation was higher in dry seeds when the moisture ratio is 3 % or lower, potentially due to reaction of free radicals with oxygen molecules (Çelik and Atak, 2017; Forster et al., 2018).

Gamma Radiation can interact with atoms and molecules and dissociates the atoms of the water molecule in the cell and causes the generation of free radicals that are the most reactive. Therefore gamma radiation increases the free radical concentration in the living cells and to create oxidative stress of plant cell (Ali et al., 2016; Hanafy and Akladious, 2018; Jan et al., 2012; Leibovitz and Siegel, 1980; Wi et al., 2005). That's why this period effects the structure of DNA and mitosis (Singh, 1983). Moreover, " these radicals can damage or change important components of plant cells and have been suggest to affect different all the morphology, anatomy, biochemistry, and physiology of plants depending on theradiation dose" (Ashraf et al., 2003).

"The determination and adjustment of the water content of the seed have a big importance . Because there is a close relationship between the effect of gamma radiation and water content of the seed. However, even minor decreases in the water content of the seed can have a very big impact on the biological effect of gamma radiation"(Atak and Atilla, 1985). Generally, minimizing the effect of oxygen enables obtaining higher mutagenic productivity (Hase et al., 2018). According to many studies seed's sensitivity to X and gamma radiation increases as the moisture ration in seed falls below 14 % (Konzak et al.,1968 ; Roy et al., 1972; Sharma and Sharma ,1986). In a study which was carried out with barley seeds, it was found out that with the fall of the moisture ratio in barley seed from 13 % down to 2 %, the damage caused by radiation increased 1-9 times more (Conger and Konzak, 1977). In a study which was conducted with soybean seeds, with the fall of moisture level from 7.75 % down to 5.1 %, the damage caused by radiation increased as well (Yalçın, 1992). In the study that Verma, (2017), carried out with the cumin plant, they presented that a high dose of gamma radiation has an effect of decreasing germination percentage. Other studies, which show resemblance to the results obtained from other plants, also exist in this subject (Grover and Khan, 2014 ; Deshmukh et al.,2018). Studies, which show that radiation generally does not effect on germination percentage, also exist (Gordeeva et al., 2018; Kakade and Borse 2017).

Spot chlorosis has been observed on the first true leaves of the M_1 seedlings that belonged to the three different soybean varieties used in this study and on which different gamma radiation was applied. The doses at which spot chlorosis was particularly apparent were 200, 250, 300 and 400 Gy.

The physiological damage that radiation causes in M_1offspring also affects the amounts of chlorophyll. Chloroplasts are also damaged in the irradiated cells, by extension, changes in the amounts of chlorophyll and spots (spot-chlorosis) are seen (Borzouei et al., 2010; El-Mouhty, 2014).

Studies, which were carried out on plants, have established that the amount of chlorophyll decreased in M_1offspring and spot-chlorosis developed in parallel with increased doses of radiation (Kumar et al., 2019 ; Yalçın, 1992).

One of the parameters, which shows responses of plants to radiation, the best is measuring the heights of seedlings. Therefore, determining the height of the plant is important in radiobiological experiments (Gaul, 1977).

It has been found out that there was a decrease compared to control in the seedling heights of the defiance, general and iraqous soybean varieties, which were used in this study, in parallel with increased doses of radiation. As a result of the regression analysis which was conducted according to the obtained results; it has been found out that doses of gamma radiation had an effect on seedling height in the three different soybean varieties, and that there was a linear relationship between seedling height and gamma radiation doses; in other words, the decrease in seedling height is affected by the change in radiation doses, and that this was significant ($p<0.05$).

In parallel with the increase in the dose of gamma radiation, the plant cannot survive or cannot reproduce even if it lives. Therefore the limits of the doses that are to be used should be determined carefully. For that, determining the (GR_{50}) mutagen dose, which reduces seedling height by 50% compared to control, is important. This value is different for each plant species (Yasmin and Arulbalachandran, 2016; Rajapandian and Dhanam, 2017)

At the end of this study, GR_{50} dose has been determined as 229 Gy for defiance variety, 248 Gy for general variety and 265 Gy for Iiaquous variety. In the study that Anuradha et al. (2017), carried out with fenugreek, they used different doses of gamma radiation and determined GR_{50} doses as 350 Gy . In research that Sarsu Demir (2003), conducted, they applied 400, 600, 800, 1000 and 1200 Gy gamma radiation on two different rape varieties. They found out that there were significant falls in traits like root length, seedling height, number of seeds, the weight of seeds compared to control, in parallel with increased dose.

In the study that Artık and Peşken, (2006), carried out, they recorded observations like time and ratio of appearance, start and end time of blooming, blooming period, first broad bean formation time, harvest ripeness time in M_1 generation of two different broad bean varieties at 0, 25, 50, 75 and 100 Gy doses of gamma radiation. They found out that appearance ratios in the

M_1 generation of the varieties that were dealt with showed increase at 25 and 50 Gy doses in the two broad bean varieties, and then decreased in parallel with an increase of dose.

In a research that Abdel-Hady and Ali (2006), conducted, they studied the effects on callus growth ratio, plant regeneration and agricultural practices in four different wheat cultures, using 150, 250, 350 and 450 Gy gamma radiation doses; they found out that radiation doses lower than 150 Gy had a stimulating effect on all the characters they studied. At the same time, they found out that it caused a decrease compared to control in all practices to a considerable extent, in parallel with the increase of high doses. It has been shown that there are many differences in the sensitivity of different plant species and of diverse varieties of the same plant species to radiation and that in some cases, they are even greater in diverse varieties of the same plant species (Afify et al., 2011).

Physiological damage can emerge due to chromosomal or extrachromosomal reasons. Increased radiation dose also increases the amount of physiological damage. Increased dose of radiation increases the amount of physiological damage too (Kumar and Srivastava, 2010). The reason for the decrease in seedling heights of defiance, general and iraquous varieties with the increased dose can be explained by the decreasing of mitosis frequency in apical meristem with the increase of dose .

Ionizing radiation applied to plant cells cause the death of cells. Loss of the ability to divide can be accepted as cell death in dividing cells. These cells' losing their ability to divide continuously is called mitotic death (Ozalpan, 2001). However, in some studies that were conducted, it was observed that a low dose of radiation made a positive effect in different characteristics in the seed (Hegazi and Hamideldin, 2010). Again, in a study that was carried out, it was observed that as gamma radiation can stimulate harmful mutations in plants as a mutagen, it can also stimulate beneficial mutations (David et al., 2018; Kusmiyati et al., 2018). Lande (2018), found more genetic variability compared to control by using gamma radiation in soybean. Similar results were obtained in researches that were done on rice and sesame plants as well (Gowthami et al.,2017 Muhammad et al., 2018).

5. Conclusion

In M_1 generation of defiance, general and iraquous soybean varieties, germination percentage and seedling heights have decreased depending on increased doses of radiation; spot chlorosis has been found in the first true leaves of M_1 seedlings.The resistance of the three different soybean varieties against radiation has been detected as iraquous (GR_{50}=265 Gy), general (GR_{50}=248 Gy) and defiance (GR_{50}=229 Gy), respectively.

List of Abbreviations: GR50: The dose that decreases growth by 50 percent; Gy:(Gray) unit of measurement for absorbed radiation. M_1: The seed is called the M_1 seed for the first generation following the mutagenesis treatment.

Competing Interest Statement: This is declared that the authors of this article do not have any competing interest.

Author's Contribution: Both the authors share equal contribution towards the present form of manuscript.

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