

To Cite: Yaman, H., & Bayraktar, N. (2023). The Effects of Different Gamma-Ray Doses on Safflower (*Carthamus tinctorius* L.) Varieties on Agricultural Features Observed in M1 and M2 Plants. *Journal of the Institute of Science and Technology*, 13(1), 670-684.

The Effects of Different Gamma-Ray Doses on Safflower (*Carthamus Tinctorius* L.) Varieties on Agricultural Features Observed in M1 and M2 Plants

Humeyra YAMAN^{1*}, Nilgun BAYRAKTAR²

Highlights:

- Plant height and head diameter of the safflower plant are affected by mutation applications.
- A regular distribution was not observed due to the increase in the amount of gamma dose.
- The response of genotypes to gamma-ray application may differ.

Keywords:

- Genetic diversity
- Ionizing radiation
- Mutation
- Seed yield
- Viability

ABSTRACT:

The research was carried out with the ionizing radiation source Cobalt 60 (Co-60) at a dosage of 200, 300, 400, 500 Gy on three safflower varieties. According to the results of the research, in M1 plants; in parallel with the increase in gamma dosages, a certain decrease occurred in plant height, number of branches per plant, number of trays per plant, diameter of the tray, number of seeds per tray, seed yield per plant, and seed vitality. In field observations taken from M2 plants, significant diversity was observed. In M2 plants, significant reductions in emergence rate were observed in all three cultivars depending on the increase in dosages. In all three safflower cultivars, plant height, tray diameter, number of seeds per tray and thousand seed weight values increased compared to control plants in parallel with the increase in dosages. Significant mutations were observed in the 200-400 Gy dosage range in all three cultivars. In M1 plants, when the seed yield per plant and the rate of viability were examined, a certain level of decrease was observed in parallel with the increase in gamma dosages. In M2 plants, in parallel with the dosage increase in all cultivars, the tray diameter values increased compared to the control. Again, in all cultivars, while the diameter of the tray decreased in M1, it increased in M2 in general and increases were observed at low gamma dosages. In M2, dosages of 300-400 Gy also increased in the criteria examined compared to the control and had a stimulating effect. The investigated properties generally decreased in M1 depending on the dosage increase, while there were fluctuations in M2. As a result, the variation created by the radiation and the real effect of the mutation applications emerged after M2.

In this study, while the averages of Dinçer and Remzibey cultivars in terms of viability in M1 were almost the same, Shifa cultivar gave more successful results in terms of viability. When the values obtained in the study were examined, it was concluded that genetic variations and changes occurred at dosages of 300-400 Gy at most.

¹Humeyra YAMAN (Orcid ID: 0000-0002-5873-9401), Ministry of Agriculture and Forestry, Field Crops Central Research Institute, Ankara, Turkey

²Nilgun BAYRAKTAR (Orcid ID: 0000-0003-0425-6305), Ankara University, Faculty of Agriculture, Department of Field Crops, Ankara, Turkey

*Corresponding Author: Humeyra YAMAN, e-mail: humeyrayaman@hotmail.com

This study was produced from Humeyra YAMAN's PhD thesis (number 10062787).

INTRODUCTION

Safflower (*Carthamus tinctorius L.*) is a member of the Compositae / Asteraceae family and is one of the oldest cultivated plants. It is also reported that there are about 25 species in the world (Singh and Nimbkar, 2006, Arslan et al., 2010). Ashri (1957), Ashri and Knowles (1960) collected the species belonging to the genus *Carthamus L.* in 4 sections in their systematic research based on cytological and morphological observations and included the species with the number of chromosomes $2n = 24$ in Section I.

Safflower production is carried out in 23 countries in the world and when 2020 data is analyzed, the first three countries with the highest cultivation area are Kazakhstan with 315.177 ha, followed by Russian Federation with 174.974 ha, and India with 85.475 ha. Turkey is in the 10th place and its cultivation area is 15.114 ha. In terms of production quantities, Kazakhstan is 226.739 tons, Russian Federation is 96.636 tons, and Mexico is 86.793 tons. In Turkey, 21.325 tons of safflower was produced in 2020.

When the safflower seed yield values of the countries are examined, according to the 2019 data; Mexico 185.6 kg da-1, China 146.8 kg da-1, USA 142.6 kg da-1, and Turkey 138.0 kg da-1, respectively (FAOSTAT, 2021). According to TUIK (2020), the largest cultivation area in Turkey belongs to 2014 with 44.305 ha. The maximum safflower production was in 2015 with 70 kilotons. The highest yield was in 2010 with 193 kg da-1. The highest seed production of safflower was 975 tons in 2017. Safflower production and cultivation decreased in 2020, and 11.115 ha cultivation area, 21.325 tons of seed production, and 141 kg ha-1 seed yield were obtained (TUIK, 2020).

Although safflower is a significant oilseed plant, its agriculture is not at the desired level in the World due to its low yield and oil rate compared to other cultivated plants. High seed yield, high oil yield, earliness, high linoleic or high oleic acid ratio are at the forefront in safflower breeding (Koç, 2019). In breeding studies, different methods can be used to achieve the desired traits, and the main goal is to provide genetic variation. This variation appears to be naturally induced. Natural mutations are the dominant source of genetic diversity in plants, and they are the cause of all genetic diversity in other organisms (Kharkwal, 2012, Oladosu et al., 2016). Several researchers mentioned that the genetic diversity in plants emerged as a result of spontaneous variation between 1590 and 1968 (Solanki et al., 2015). The first publications of induced mutations (X-ray rays) for plant breeding were published in 1928 by Muller and Stadler (Beyaz and Yıldız, 2017).

Mutation breeding aims to obtain new varieties that are highly productive, adaptive to growing conditions, having superior quality characteristics, and are resistant to diseases and pests. Changes in the number and structure of chromosomes and the changes in genes are named mutations. In the FAO/IAEA mutant cultivar list, there are 2252 cultivars before 2000. The number of cultivars developed by mutation was 175 in 2000. (Karakoca and Akgün, 2020). In 2017, there are 3246 registered mutagenic plant varieties in the FAO/IEAE mutant database (Beyaz and Yıldız, 2017).

Gamma rays cause changes in molecules or atoms. Post-irradiation may cause physiological and anatomical differences in the plant. The change in plant cellular structure and functioning mechanism can be observed morphologically. In addition, it can form free radicals in the plant and affect important components (Ertem Vaizoğullar et al., 2016). For this reason, it is significant to select the administered dosages with precision. For this reason, it is significant to select the administered dosages with precision. In the studies conducted, it was mentioned that features such as germination, sprouting, plant height, root length, biomass were promoted by using a ^{60}Co gamma radiation source at certain

doses to trigger genetic variability in sunflower. However, they also reported that these properties may decrease with increasing doses (Diaz et al. 2018, Yalçın and Ulakoglu. 2019)

In mutation breeding, the first target is to reach the mutation frequency where the minimum damage to the plant originating from the radiation and a high yield are achieved. It is necessary to select the mutagen dosage and application methods in accordance with the purpose, to observe the changes in the plants, and to determine the resulting physiological damages quantitatively (Yazıcı et al., 2016). For this purpose, an irradiation study was carried out to determine the radiation dosages in safflower optimally, and some agricultural characteristics were recorded.

MATERIALS AND METHODS

The research was carried out with Remzibey, Dinçer, and Shifa safflower varieties in the Gölbaşı-İkizce Research and Application Station of the Field Crops Central Research Institute. The moisture rate in the seeds of these safflower cultivars was adjusted to 10-11%. (Tarighi et al., 2010). The seeds were placed in the envelopes and placed in the Co-60 source, where they were exposed to different doses of radiation. The plants were irradiated at dosages of 200, 300, 400, 500, and 600 Gy (Co-60 dosage rate 0.549 kGy hr⁻¹) using the Cobalt 60 source at the Turkish Atomic Energy Agency, Sarayköy Nuclear Research Institute. Also, non-irradiated seeds were included in the study to represent the control.

The climatic conditions of the research area; while the total rainfall was 379.9 mm in the 2009-2010 growing season, the highest temperature was 35°C in July, and the lowest temperature was -13.8°C in January 2010. Since the precipitation period is in the winter season, the yields were recorded as low. In the 2010-2011 growing season, the total rainfall is 401.6 mm, the highest temperature is 39°C in August, and the lowest temperature is -18.2°C in February 2011. Precipitation occurred during the development period.

The soil of the research area is clayey-loam, alkaline, calcareous, has no salt problem, contains a certain amount of plant-available phosphorus, is rich in potassium content, and is poor in organic matter and nitrogen content.

The irradiated seeds were sown in randomized blocks in split plots design with three replications. The experiment was organized in such a way that varieties were in the main plots, and dosages were in the sub-plots. In autumn, the field soil was plowed deeply (25 cm), and the area tested was left in small clods. Later, this area was plowed as an outcrop (15 cm) in the spring and the seedbed was prepared by passing the floor with a disc harrow. In the trials established in both years, sowing was done in four rows with a total plot length of 5 m, in total 100 seeds from each dosage of each variety were arranged so that the row spacing was 50 cm and the row spacing was 20 cm.

In general, genetically; While alleles are Aa in M1 generation, AA, Aa and aa in M2 generation with inbreeding gametes with alleles are formed. Gametes carrying aa recessive homozygous alleles, mutant creates types. Recessive gene mutations occur in the M2 generation. Here, M1 plants represent individuals obtained after the 1st irradiation, and M2 plants represent individuals obtained as a result of sowing seeds of M1 plants.

Maintenance and observations were made on the plants, and the main trays were isolated to prevent foreign pollination during the flowering phase. Plants at harvest maturity were harvested separately according to the application groups, and the main capsule of each plant was kept to form M2 plants (Tonnemaker et al., 1992). 0.5 m from the top and bottom of the plots and one row from each side were separated as edge effects.

With the data obtained from the research, variance analysis of the cultivars was performed separately. For the statistical evaluation of the research results, Düzgüneş et al., (1987), was used. A probability level of 0.05 was used to identify different groups in the significance tests. All calculations were made using the MSTAT-C computer package program.

RESULTS AND DISCUSSION

For M1 and M2 plants, the time from sowing to the emergence of 80% of the seedlings was taken as 13 and 15 days, and the time from sowing to the bolting of 50% of the seedlings was determined as 46 and 31 days, respectively. Esendal (1973), worked with wild and cultivated safflower, recorded the time that the plants took root separately according to the varieties, and stated that this period varied between 42-52 days. Emergence and bolting times may vary due to genotypic and ecological differences and mutagen applications used in studies.

For M1 and M2 plants, the time from sowing to the date of the first flower tray was found as 74 days. In addition to the changes in the place and climatic conditions where the experiments are carried out, it is thought that the biological and therefore physiological effects of mutagen applications and the differences in the varieties are very important. The changes made by different mutagen dosages on the genetic structure of the plant are reflected positively or negatively on the plant development. The time from sowing to harvest maturity for M1 and M2 plants was 126 and 139 days, respectively. However, the time from sowing to harvest maturity in the control plants used in the experiment was 121-125 days for the first and second years, respectively.

Observations and Measurements of M1 Plants

According to the data obtained from M1 plants; different cultivars are statistically significant at the 1% level in terms of plant height, and different dosages of gamma rays and interaction (variety \times gamma ray dosage) are insignificant. Different cultivars, different dosages of gamma ray application and interaction (variety \times gamma ray dosage) are statistically insignificant in terms of the number of branches and the number of stems per plant. Different varieties and different dosages of gamma rays are statistically significant at the level of 1% in terms of tray diameter and thousand seed weight, while the interaction (variety \times gamma ray dosage) is insignificant. Different cultivars and interaction (variety \times gamma ray dosage) are statistically insignificant in terms of the number of seeds in the tray, while the application of different dosages of gamma rays is significant at the 1% level. In terms of seed yield per plant, different varieties, application of different dosages of gamma rays and interaction (variety \times gamma ray dosage) are statistically significant at the level of 1%. In terms of the viability, different varieties, different dosages of gamma ray application were statistically significant at the level of 1%, and interaction (variety \times gamma ray dosage) was statistically significant at the level of 5%.

The height of 20 randomly selected plants according to the application groups was measured and recorded from the root collar to the point where the stem was attached to the tray. The plant height varied between 34.56-68.88 cm, and different cultivars formed three groups according to gamma-ray dosage. The number of branches changed between 6.32 and 8.26 depending on the cultivars at different gamma-ray dosages. The number of trays per plant varied between 12.63-20.22. The diameter of the tray changed between 1.69-2.66 cm and 3 groups were formed in different varieties with different dosages. 300-400 Gy dosages placed in the same group. The thousand-seed weight varied between 42.06-64.56 g, and as the gamma dosages increased, the thousand-seed weight also increased. Dosages of 200-400 Gy were placed in the same group (Table 2).

The Effects of Different Gamma-Ray Doses on Safflower (*Carthamus Tinctorius* L.) Varieties on Agricultural Features Observed in M1 and M2 Plants

Table 1. Analysis of variance of the parameters examined in M1 plants of different doses of gamma rays in safflower cultivars

M1 plants/parametres	Plant height (cm)	Number of branches per plant (pcs)	Number of trays per plant (pcs)	Tray diameter (cm)	Number of seeds per tray (pcs)	Thousand-seed weight (g)	Seed yield per plant (g)	Viability (%)	
Variation source	Degrees of Freedom	Mean of Squares							
Recurrence	2	0.607	0.393	6.653	0.020	46.799	22.76	0.413	7.351
Variety (A)	2	2348.85**	2.984	26.94	1.714**	187.48	446.24**	69.016**	441.74**
Error 1	4	100.57	0.997	5.449	0.029	87.558	2.834	0.835	9.89
Dosage (B)	4	26.619	0.503	11.35	0.123**	758.18**	114.32**	211.45**	588.89**
AxB	8	17.892	0.633	11.88	0.024	41.497	32.17	11.764**	45.99*
Error 2	24	35.901	0.762	7.408	0.022	35.450	19.97	1.615	14.11
Toplam	44	141.19	0.821	9.253	0.108	114.42	48.71	25.474	90.91

* 0.05, ** 0.01 important at the probability level

Table 2. Duncan test results of the effect of different dosages of gamma rays in Safflower cultivars in M1 plants

Parameters	1 st year observations and measurement						CV (%)
	Cultivars	Gamma-ray dosages (Gy)					
Plant height (cm)	Cultivars	0	200	300	400	500	11.29
	Remzibey	41.90	41.52	39.04	38.69	34.56	
	Dinçer	55.76	55.00	57.98	58.25	56.00	
Number of branches per plant (pcs)	Shifa	68.88	62.39	63.23	60.92	61.60	11.88
	Remzibey	7.66	6.82	7.07	6.77	6.32	
	Dinçer	7.51	7.42	7.68	8.19	8.26	
Number of trays per plant (pcs)	Shifa	8.07	7.15	7.06	6.98	7.20	16.82
	Remzibey	15.85	13.96	12.63	16.51	14.63	
	Dinçer	17.55	14.75	16.50	19.14	18.80	
Tray diameter (cm)	Shifa	20.22	16.89	16.79	14.55	13.91	7.04
	Remzibey	1.80	1.79	1.73	1.71	1.69	
	Dinçer	2.29	2.15	2.09	2.21	1.90	
Number of seeds per tray (pcs)	Shifa	2.66	2.55	2.38	2.28	2.22	23.23
	Remzibey	30.07	22.23	23.21	20.93	12.30	
	Dinçer	41.08	25.30	25.20	26.36	14.30	
Thousand-seed wight (g)	Shifa	46.76	31.55	29.72	20.59	14.76	8.63
	Remzibey	42.06	47.21	42.36	46.43	51.86	
	Dinçer	47.00	54.91	52.33	57.40	51.03	
Seed yield per plant (g)	Shifa	49.78	56.25	57.56	55.93	64.56	11.50
	Remzibey	15.56b	10.65cd	8.70def	8.06efg	4.46h	
	Dinçer	15.46b	12.16c	8.80def	7.34efg	7.06fg	
Viability (%)	Shifa	24.11a	16.09b	11.93c	9.37de	5.97gh	4.34
	Remzibey	95.88ab	89.60bcd	86.02d	71.46f	70.41f	
	Dinçer	93.99ab	89.71bcd	86.79cd	78.82e	71.30f	
	Shifa	98.14a	94.76ab	93.04abc	90.47bcd	87.16cd	

According to the data obtained from M1 plants, some characters were found to be statistically significant. Different varieties in terms of plant height is 1%, different varieties and different doses of gamma rays in terms of tray diameter are 1%, different doses of gamma rays in terms of the number of seeds per tray is 1%, different varieties and different doses of gamma rays in terms of thousand seed weight are 1%, different varieties, different doses of gamma ray and interaction (variety x gamma-ray dosage) in terms of seed yield per plant are 1% and different varieties, different doses of gamma ray in terms of vitality are 1%, interaction (variety x gamma-ray dosage) is found significant at the 5% level (Table 1).

Seed yield per plant varied between 4.46-24.11 g, and different cultivars formed two groups, and different dosages formed five groups. While Shifa 0 (Control) had the highest seed yield with 24.11 g,

The Effects of Different Gamma-Ray Doses on Safflower (*Carthamus Tinctorius L.*) Varieties on Agricultural Features Observed in M1 and M2 Plants

Remzibey 500 Gy had the lowest yield with 4.46 g (Table 2). There was a linear decrease in seed yield in all three cultivars depending on the dosage increase. However, the highest yield decrease is in the Shifa variety. Considering the seed yield averages, the highest yield among the cultivars was obtained from the Shifa cultivar. Control data of the Shifa cultivar were higher than other cultivars in terms of the number of trays per plant and the number of seeds per tray. In this case, the seed yield will inevitably be high in the plant. According to the application groups, the trays of the harvested plants were separated, and the seeds were removed and weighed on a precision scale to calculate the seed yield per plant.

Considering the seed yields per plant, it was seen that the Shifa cultivar is affected by a linear decrease in yield from irradiation in the first year. In addition, Dinçer and Remzibey cultivars had a lower seed yield than the Shifa cultivar (Figure 1).

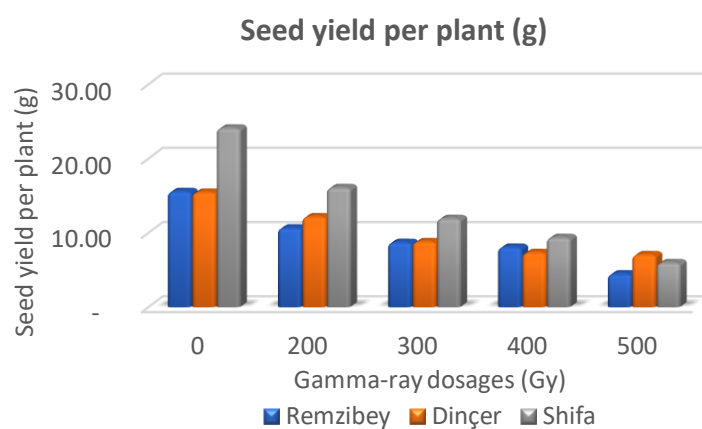


Figure 1. The effect of different dosages of gamma rays on seed yield per plant in M1 safflower cultivars

The viability rate varied between 70.4% and 98.14%, and different cultivars formed two groups, and different dosages formed four groups. 200 and 300 Gy dosages placed in the same group. While the highest rate was obtained from the three cultivars in control with 93.99%-98.14%, the lowest rate was observed in Remzibey and Dinçer 500 Gy with 70.4% and 71.30%, respectively (Table 2). As seen in the Dinçer cultivar, no healthy growth could be obtained in any cultivar at 600 Gy dosages, and even if there were individual emergences in the field, the plants could not survive for a long time. Compared to the other two cultivars, the decrease in viability of the Shifa cultivar was less with compared to the other cultivars due to the increase in dosage.

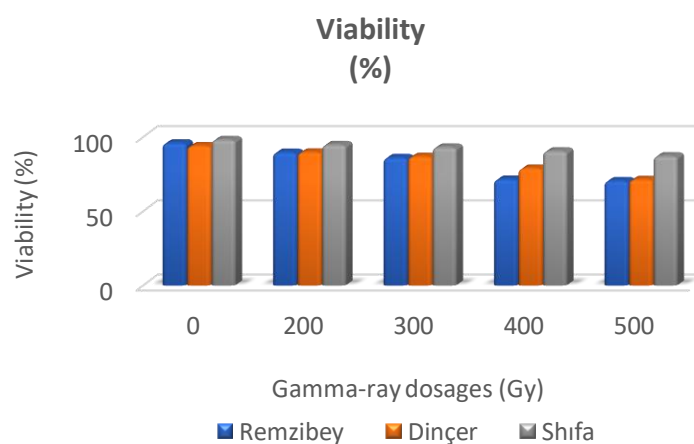


Figure 2. The effect of different dosages of gamma rays on the viability of M1 plants in safflower cultivars

The Effects of Different Gamma-Ray Doses on Safflower (*Carthamus Tinctorius L.*) Varieties on Agricultural Features Observed in M1 and M2 Plants

A more stable decrease in the viability of the Shifa cultivar was observed compared to other cultivars. The effect of the mutagen source applied in all cultivars resulted in a decrease in viability because of the genetic change (Figure 2).

Observations and Measurements of M2 Plants

According to the data obtained from M2 plants; Different cultivars are statistically significant at the 5% level in terms of plant height, the number of seeds per tray and thousand seed weight, and different dosages of gamma-ray application and interaction (variety x gamma-ray dosage) are insignificant. Different varieties, different dosages of gamma rays, and interaction (variety x gamma-ray dosage) were statistically insignificant in terms of the number of branches per plant and seed yield per plant. Different cultivars are statistically significant at the 1% level in terms of the number of trays in the plant, and different dosages of gamma rays and interaction (variety x gamma-ray dosage) are insignificant. In terms of tray diameter, different varieties and different dosages of gamma rays were statistically significant at the 1% level, and the interaction (variety x gamma-ray dosage) was significant at the 5% level.

Plant height varied between 64.43-87.20 cm, and different cultivars formed two groups. Plant height did not change according to different gamma-ray dosages. Here the difference between the varieties is significant, but the dosages and interaction are negligible. The number of branches in the plant was between 5.96-7.86. The number of trays varied between 8.90-17.46, and different varieties formed two groups. The Remzibey cultivar has the highest number of trays with 16.92 trays. There was no significant difference between the number of trays of the other two cultivars (Table 4).

Table 3. Analysis of variance of the parameters examined in M2 plants of different doses of gamma rays in safflower cultivars

M2 plants/parametres		Plant height (cm)	Number of branches per plant (pcs)	Number of trays per plant (pcs)	Tray diameter (cm)	Number of seeds per tray (pcs)	Thousand-seed weight (g)	Seed yield per plant (g)
Variation source	Degrees of Freedom	Mean of Squares						
Recurrence	2	68.85	11.82	120.25	0.045	41.81	8.79	134.29
Variety (A)	2	1202.34*	5.10	206.42**	1.658**	724.05*	453.09*	54.66
Error 1	4	108.97	1.66	9.58	0.030	61.37	36.99	12.24
Dosage (B)	4	5.05	0.23	3.29	0.059**	85.12	12.50	12.31
AxB	8	14.32	0.34	2.93	0.018*	23.47	10.65	8.19
Error 2	24	17.89	0.57	3.17	0.006	36.85	13.63	9.26
Toplam	44	80.51	1.31	18.28	0.092	72.50	34.87	17.36

* 0.05, ** 0.01 important at the probability level

According to the data obtained from M2 plants, some characters were found to be statistically significant. Different varieties in terms of plant height is 5%, different varieties in terms of number of trays per plant is 1%, different varieties and different doses of gamma rays in terms of tray diameter are 1%, interaction (variety x gamma-ray dosage) in terms of tray diameter is 5%, different cultivars in terms of number of seeds per tray is 5%, and different cultivars in thousand seed weight is found significant at the 5% level (Table 3).

Tray diameter varied between 1.60-2.53 cm. Different varieties and different dosages formed two groups. Dosages of 0-300-400 Gy and 200-500 Gy were included in the same group. The highest tray diameter was obtained from 2.53 cm and 200 Gy dosage and from 2.50 cm to 500 Gy dosage in the Shifa cultivar.

The Effects of Different Gamma-Ray Doses on Safflower (*Carthamus Tinctorius* L.) Varieties on Agricultural Features Observed in M1 and M2 Plants

The lowest tray diameter was obtained from 0-300-400 Gy in the Remzibey cultivar with 1.60-1.66 cm (Table 4). Increases of 200 Gy for Remzibey and Shifa cultivars and 300 Gy for Dinçer cultivars were significantly different from other increases and control. In all three cultivars, there were increases in the dosage of 500 Gy and the control tray diameters narrowed in all three cultivars compared to the first year. The average tray diameter of all three cultivars in M2 generation was high at 200 Gy dosage. In addition, the average tray diameter of the Shifa cultivar was higher than other cultivars. The difference between the minimum and maximum values of the tray diameter in the plants in the radiation applications was higher than the control. In other words, there is a positive correlation between these two criteria.

Table 4. Duncan test results of the effect of different dosages of gamma rays in Safflower cultivars in M2 plants

Parameters	2nd year observations and measurement						CV (%)
	Cultivars	Gamma-ray dosages (Gy)					
Plant height (cm)	Remzibey	65.83	64.76	64.43	66.99	67.42	5.65
	Dinçer	75.84	72.06	76.23	76.68	73.42	
	Shifa	82.76	87.20	83.96	84.25	80.78	
Number of branches per plant (pcs)	Remzibey	7.86	7.43	7.83	7.46	7.33	10.74
	Dinçer	5.96	6.53	6.80	6.30	6.50	
	Shifa	7.20	7.26	6.80	6.43	7.33	
Number of trays per plant (pcs)	Remzibey	17.43	16.06	17.46	17.13	16.50	13.76
	Dinçer	10.83	11.26	13.56	11.50	14.40	
	Shifa	10.00	9.50	9.53	8.90	9.96	
Tray diameter (cm)	Remzibey	1.66gh	1.80f	1.60h	1.66gh	1.76fg	3.88
	Dinçer	2.10de	2.10de	2.13cde	2.03e	2.13cde	
	Shifa	2.23bc	2.53a	2.33b	2.20cd	2.50a	
Number of seeds per tray (pcs)	Remzibey	17.75	19.07	19.27	20.39	22.77	22.27
	Dinçer	28.38	25.52	23.88	32.54	31.07	
	Shifa	32.68	29.12	32.10	31.61	42.65	
Thousand-seed weight (g)	Remzibey	36.38	34.04	36.15	40.38	37.203	8.69
	Dinçer	44.21	40.53	44.74	42.39	42.39	
	Shifa	47.07	46.97	47.08	46.92	50.99	
Seed yield per plant (g)	Remzibey	9.77	8.38	10.62	11.56	11.73	24.22
	Dinçer	13.16	11.44	15.07	11.64	14.80	
	Shifa	16.38	14.22	12.57	11.22	15.88	

Considering the tray diameter values in the second year, the Shifa cultivar had larger trays with respect to other cultivars. Tray diameter remained stable in the Dinçer cultivar, while differences in diameter sizes were observed in the Remzibey cultivar according to the applied dosages (Figure 3).

The number of seeds per tray varied between 17.75-42.65, and different varieties formed two groups. Thousand-seed weights varied between 34.04-50.99 g, and different cultivars formed two groups. Seed yield per plant changed between 8.38-16.38 g (Table 4).

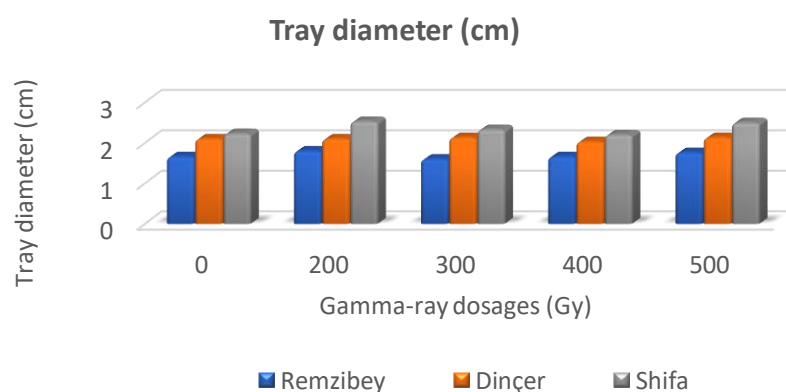


Figure 3. The effect of different dosages of gamma rays on the tray diameter of M2 plants in safflower cultivars

DISCUSSION

The responses of ionizing radiation applications under field conditions were examined, and successful results were obtained regarding the positive effect of gamma-ray dosages on these properties.

Mostly, the same characters were examined in M1 and M2 plants in the field studies. However, the investigated characters gave different results in M1 and M2 depending on the dosages. The reason for this is chromosome mutations in M1 and later generations, and mostly recessive gene mutations in M2 generations. The next step is to decide whether the change is a mutation or a modification (Başer et al., 2007).

As the mutagen dosage increases in breeding studies, the mutation frequency also increases, and as a result, genetic damage in plant genes and changes in plant functions occur. For this purpose, Ankara Sarayköy Nuclear Research and Training Center Nuclear Agriculture Department examined the appropriate mutation dosage for Dinçer, Remzibey, and Yenice cultivars. As a result, they found that the recommended effective dosage for mutation studies was between 250-400 Gy (Sagel et al., 2002). As a result of the observations, the mutation frequency increases as a result of the application of 200-400 Gy dosages, and the desired characteristics can be obtained.

In 2008, Jagadeesan et al., applied gamma rays to sunflowers and observed the plants for two years. Physical changes of gamma radiation were observed in M1 plants at different levels according to the cultivars. Stimulant and inhibitory effects were noticed in quantitative characters with increasing dosages. Quantitatively, the mean expression and variability were in M2 plants and increased significantly compared to M1 plants in the traits studied. Different mutagen dosages showed an inconsistent association for some characters in cultivars. However, the researchers noted a significant increase in variation. They found that genetic improvement in traits such as plant height, seed yield per plant, and oil content reached the highest level at 20 Kr. However, in both varieties, they recommended the appropriate dosage as 5 Kr. The data obtained in the study confirm that the mutagen dosages applied in safflower cultivars have a reducing effect in the first year and that the change and variation in the characters are more pronounced in the following year.

In M1 plants, plant height in Remzibey and Shifa cultivars was decreased in general, although not significantly. However, in the Dinçer cultivar, it was observed that plant height increased at 400 Gy dosages. An increase was observed at 300 and 500 Gy dosages compared to the control. In addition, plant height increased in M2 plants at a dosage of 400 Gy. Andrew-Peter-Leon et al., (2021), treated the White Ponni rice seeds with different dosages of gamma-ray (100, 200, 300, 400, and 500 Gy). As a result, they noted that gamma-ray caused the shortening of plants in height.

The Effects of Different Gamma-Ray Doses on Safflower (*Carthamus Tinctorius L.*) Varieties on Agricultural Features Observed in M1 and M2 Plants

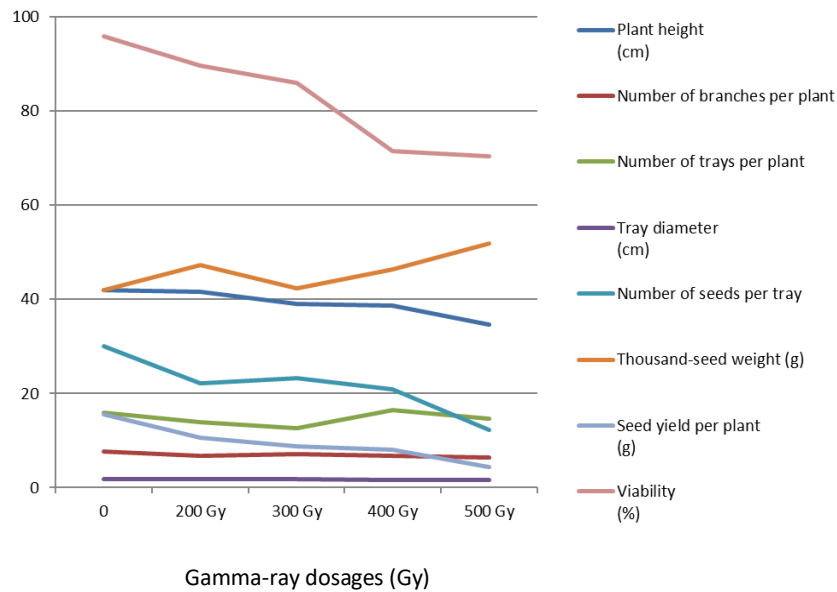


Figure 4. The effect of different dosages of gamma-ray in M1 plants of the Remzibey cultivar

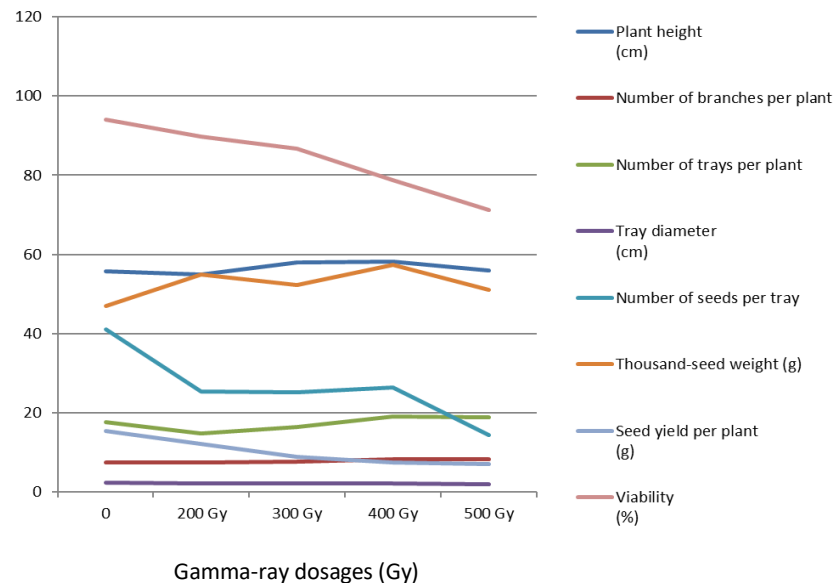


Figure 5. The effect of different dosages of gamma-ray in M1 plants of the Dinçer cultivar

In the field observations of M1 plants, when the seed yield and viability rate per plant in some cultivars were examined, decreases were observed in parallel with the increase in gamma dosages (Figure 4,5,6). In Remzibey and Shifa cultivars, the viability decreased at increasing dosages, the lengths of the plants were shortened, there was no statistical change in the diameter of the tray, and the number of seeds per tray decreased. The seeds were coarser-grained. Seed yield per plant also decreased. Here, the coarsening of the grains increased the crust ratio and appeared as a decrease in weight (Figure 4,6). The viability of the Dinçer cultivar decreased, and the number of seeds per tray and seed yield per plant decreased in live plants (Figure 5). Madibu et al., (2012), reported that increasing gamma rays decreased the values of some agricultural properties of soybean. Sagel et al., (2013), stated that increased gamma-ray dosages in M1 of chickpea had a negative effect on emergence rate, seedling height, root length, and dry weight. All mentioned studies and this research found a parallel decrease due to the increase in dosages in M1 plants. Because with radiation

application, events such as chromosome damage, slowing of the cell cycle, and delay of mitosis occur. Thus, plant regeneration and development are significantly reduced at high dosages.

In the field observations of M1 plants, the seed yield and viability rate per plant in some cultivars decreased in parallel with the increase in gamma dosages (Figure 4, 5, 6). In 2008, Jagadeesan et al., found that there was a decrease in viability due to the increase in dosage in the first year in sunflowers, and they associated this with the biological status of the plant. However, they revealed that the linear relationship between dosage and genotypic variance was not preserved in the M2 generation. The increase observed for all characters in the genotypes examined varied, and they also recorded inconsistent associations between the characters observed in the variety. Plant height, tray diameter, seed yield per plant, and oil content were the characteristics that show the most variability. In this study, there was a statistically insignificant increase in the number of trays, but there were statistically significant decreases in yields since seed-filled trays were selected.

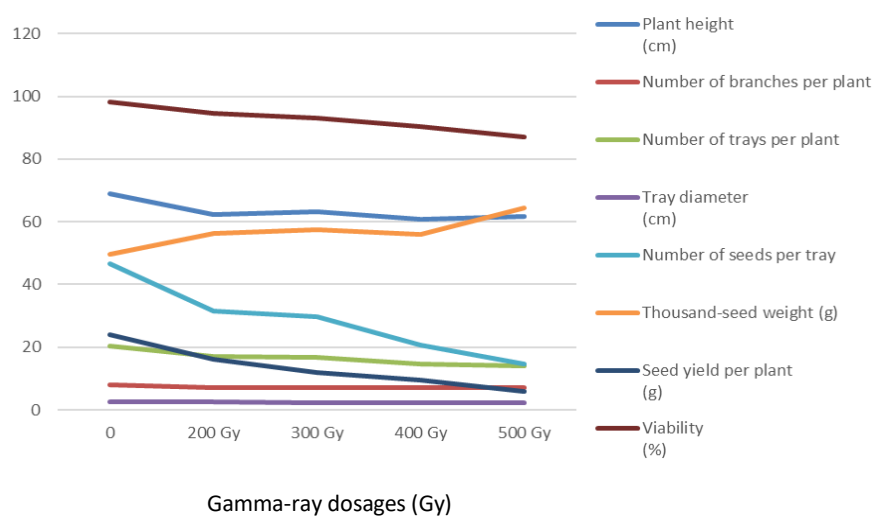


Figure 6. The effect of different dosages of gamma-ray in M1 plants of the Shifa cultivar

Field observations of M2 plants showed variation. Considering that each variety has a unique genetic structure and morphology, and therefore adaptation to different conditions, changes in observations are inevitable. In M2 plants, as the gamma-ray dosages increased in all three cultivars, statistically, the tray diameter values also increased compared to the control in parallel with the dosage increase.

Andrew-Peter-Leon et al., (2021), treated White Ponni rice seeds with different dosages of gamma irradiation (100, 200, 300, 400, and 500 Gy). They found that gamma irradiation had both positive and negative correlations on the traits they examined. For example, they found that there was a positive correlation between 50% flowering and plant height, and that flowering was also negatively correlated in some traits.

In M2 plants, although there was no significant difference in the number of seeds per tray in Remzibey and Shifa cultivars, there was an increase in the seed yield per plant and thousand-seed weight (Figure 7, 9). In the Dinçer cultivar, an increase in seed yield per plant was observed with an increase in the number of seeds per tray (Figure 8). There is a positive correlation between these examined features.

The Effects of Different Gamma-Ray Doses on Safflower (*Carthamus Tinctorius L.*) Varieties on Agricultural Features Observed in M1 and M2 Plants

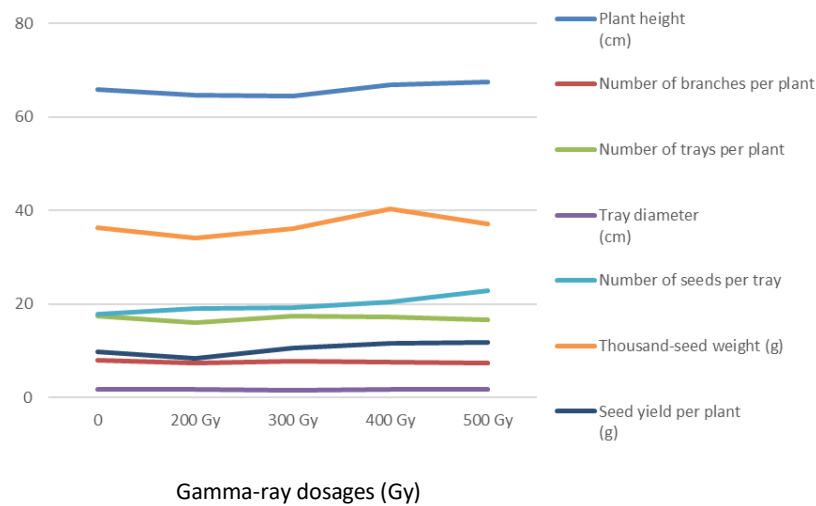


Figure 7. The effect of different dosages of gamma-ray in M2 plants of the Remzibey cultivar

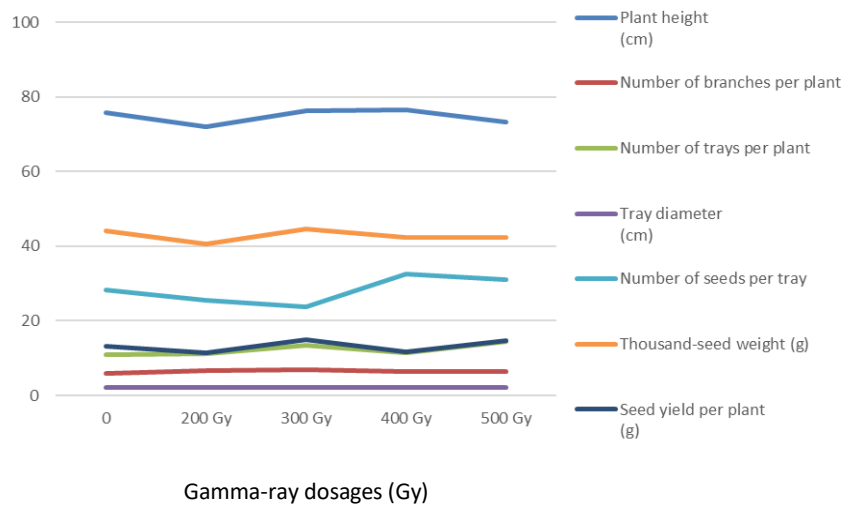


Figure 8. The effect of different dosages of gamma-ray in M2 plants of the Dinçer cultivar

Adewusi et al., (2021), applied nine different Co -60 gamma irradiations (0, 50, 100, 150, 200, 250, 300, 350, and 400 Gy) to the seeds of rice cultivars. They stated that various effects of gamma irradiation appeared on the traits. It was found that there was no decrease in the properties examined until 300 Gy application, but at 350 Gy, the values of properties such as plant height, lodging density, the number of leaves, leaf length, and leaf angle decreased significantly in both generations (M1 and M2) compared to the control. In this study, the values showed a decrease after 400 Gy dosage in general terms according to the cultivars.

The Effects of Different Gamma-Ray Doses on Safflower (*Carthamus Tinctorius L.*) Varieties on Agricultural Features Observed in M1 and M2 Plants

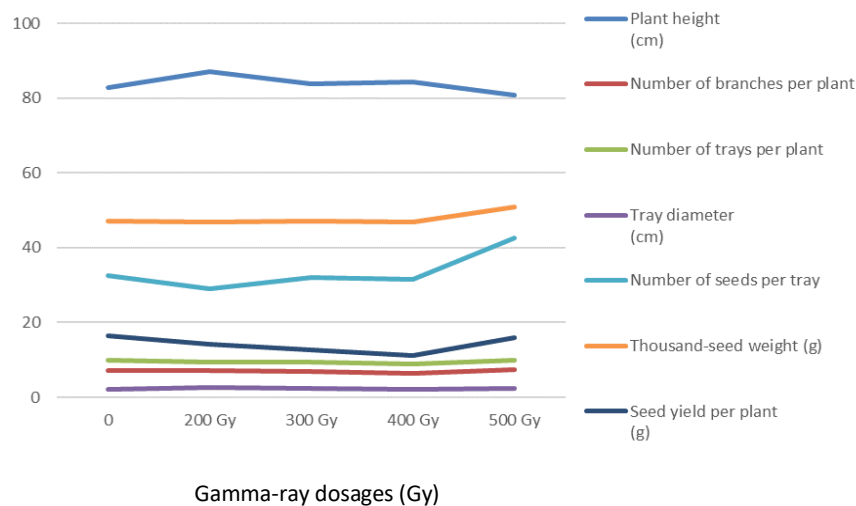


Figure 9. The effect of different dosages of gamma-ray in M2 plants of the Shifa cultivar

In this study, while the plate diameter decreased in M1 in all three cultivars, it increased in M2 in general, and increases were observed at low gamma dosages. Madibu et al., (2012), examined the same characteristics in both generations and obtained different results in some of the traits in each generation compared to the other. In M2, 300-400 Gy dosages also showed an increase in the traits compared to the control and had a stimulating effect. The traits generally decreased in M1 depending on the dosage increase, while there were fluctuations in M2. The real effect of mutation applications emerged after M2. In addition, it can be said that the harmful effect of gamma radiation in M1 disappeared in M2. For this reason, they noted that high-dosage applications also negatively affected the mutation. For this reason, in this study, mutations were examined in the second year.

CONCLUSION

The main purpose of mutation breeding is to reveal the variations and to select the desired traits among these variations. As a result of this selection, the breeder can obtain new cultivars from the mutagen lines whose yield and quality traits are examined. Here, it is necessary to reveal the physiological damage in M1 plants so that the breeder can choose the mutagen dosages and applications appropriately.

In the traits investigated in this study, fluctuations occurred with the increase in dosage, especially in M2 plants. For example, it can be predicted that these fluctuations in plant height, tray diameter, and other parameters are the result of breaks in chromosomes. A regular distribution was not observed depending on the increase in the dosage amount.

The difference between all three cultivars in field studies in terms of viability in M1 showed that the responses of the cultivars to gamma-ray were different. While the averages of Dinçer and Remzibey cultivars were almost the same, the Shifa cultivar gave more successful results in terms of viability. This is due to the fact that each variety has a different genetic structure.

When all the data obtained were evaluated, the most genetic diversity and changes were observed at dosages of 300-400 Gy. Seeds from plants obtained at these dosages were used in breeding studies to increase variation. Since these dosages are in the direction of increasing variation, they will also shed light on the breeder for new safflower mutation breeding studies.

ACKNOWLEDGEMENTS

We would like to thank the General Directorate of Agricultural Research and Policies, the Ministry of Agriculture and Forestry, which funded this study within the scope of the project.

Conflict of Interest

The article authors declare that there is no conflict of interest between them.

Author's Contributions

The authors declare that they have contributed equally to the article.

REFERENCES

- Adewusi, K. M., Showemimo, F. A., Nassir, A. L., Olagunju, S. O., Porbeni, J. B. O., Amira, J. O., & Aderinola, A. P. (2021). Assessment of ^{60}Co gamma radiation on early phenological stages of two generations of *OFADA* rice. *Agro-Science*, 20(1), 31-37. <https://dx.doi.org/10.4314/as.v20i1.6>
- Andrew Peter Leon, M. T., Ramchander, S., Kumar, K., Muthamilarasan, M., & Arumugam Pillai. M. (2021). Assessment of efficacy of mutagenesis of gamma-irradiation in plant height and days to maturity through expression analysis in rice. *PloS one*, 16(1), e0245603. <https://doi.org/10.1371/journal.pone.0245603>
- Arslan, Y., Katar, D., Güneylüoğlu, H., Subaşı, İ., Şahin, B., & Bülbül, A. S. (2010). The wild species of *Carthamus L.* in natural flora of Turkey and possibilities of using safflower breeding. *Journal of Field Crops Central Research Institute*, 19(1-2), 36-43. Retrieved from <https://dergipark.org.tr/en/pub/tarbitderg/issue/11502/137005>
- Ashri, A. (1957). Cytogenetic and Morphology of *Carthamus L.* Species to several foliage diseases in Israel. *Plant Dis. Rep*, 45, 146-150.
- Ashri, A., & Knowles, P. F. (1960). Cytogenetics of Safflower (*Carthamus L.*) Species and Their Hybrids. *Agronomy Journal*, 52(1), 11-17. <https://doi.org/10.2134/agronj1960.00021962005200010004x>
- Bağcı, M., & Mutlu, H. (2011). Determination of proper gamma radiation (^{60}Co) dose in mutation breeding of sainfoin (*Onobrychis sativa* Lam.). *BİBAD, Biyoloji Bilimleri Araştırma Dergisi*, 4(2), 141-144.
- Başer, İ., Bilgin, O., Korkut, K. Z., & Balkan, A. (2007). Improvement of some quantitative characters by mutation breeding in durum wheat. *Journal of Agricultural Science*, 13 (4), 346-353. https://doi.org/10.1501/Tarimbil_0000000392
- Beyaz, R., & Yildiz, M. (2017). The use of gamma irradiation in plant mutation breeding. In Snježana Jurić (Eds.), *Plant engineering* (p-p 33-46). <http://dx.doi.org/10.5772/intechopen.69974>
- Díaz, L. E., García, S. A. L., Morales, R. A., Báez, R. I., Pérez, V. E., Olivar, H. A., Vargas, R. E. J., Hernández, H. P., De la Cruz, T. E., & Loeza, C. J. M. (2018). Effect of gamma radiation of ^{60}Co on sunflower plants (*Helianthus annuus L.*) (*Asteraceae*), from irradiated achenes. *Scientia Agropecuaria*, 9(3), 313-317. <http://dx.doi.org/10.17268/sci.agropecu.2018.03.02>
- Düzgünes, O., Kesici, T., Kavuncu, O. and Gürbüz, F. (1987) Researches and Practice Methods (Statistical Methods II). A. U. Agricultural Faculty Publishes: 1021, Ankara, 381.
- Ertem Vaizoğullar, H., Kara, Y., Kuru, A. & Parlak, B. (2016). The comparison of effects of gamma radiation of crude oil yield on some sunflower (*Helianthus annuus*) seeds. *International Journal of Secondary Metabolite*, 3(1), 14-20. <https://doi.org/10.21448/ijsm.240698>
- Esendal, E. (1973). A Study on Phonological and Morphological Characteristics, Yields and Seed Properties of Some Domestic and Foreign Safflower (*Carthamus tinctorius L.*) Varieties Cultivated in Erzurum Ecological Conditions. *Atatürk University Journal of the Faculty of Agriculture*, 3(3).
- Jagadeesan, S., Kandasamy, G., Manivannan, N., & Muralidharan, V. (2008). Mean and Variability Studies in M₁ and M₂ Generations of Sunflower (*Helianthus annuus L.*). *Helia*, 31(49), 71-78. <https://doi.org/10.2298/hel0849071j>
- Justin, M., Kabwe, K. C., Adrien, K. M., & Roger, V. K. (2012). Effect of gamma irradiation on morpho-agronomic characteristics of soybeans (*Glycine max L.*). *American Journal of Plant Sciences*, 3(3), 331-337. <https://doi.org/10.4236/ajps.2012.33039>
- Karakoca, A.T., & Akgün, İ. (2020). Determination of the Mutagenic Effect of Different Gamma Radiation Doses Applications on Some Agricultural Characteristics of Barley in M₂ Generation. *Journal of Suleyman Demirel University, Graduate School of Natural and Applied Sciences*, 24(1), 96-104. <https://doi.org/10.19113/sdufenbed.580095>

The Effects of Different Gamma-Ray Doses on Safflower (*Carthamus Tinctorius L.*) Varieties on Agricultural Features Observed in M1 and M2 Plants

- Kharkwal, M. C. (2012). A brief history of plant mutagenesis. In *Plant mutation breeding and biotechnology* (pp. 21-30). Wallingford UK: CABI. <https://doi.org/10.1079/9781780640853.0021>
- Koç, H. (2019). Evaluation of Safflower Cultivars in Terms of Yield and Oil Ratio under Different Precipitation. *Turkish Journal of Agricultural and Natural Sciences*, 6(3), 518-526. <https://doi.org/10.30910/turkjans.595371>
- Madibu, J., Kabwe, K.C., Nkongolo Kalonji-Mbuyi, A., & Roger, V.K. (2012). Effect of Gamma Irradiation on Morpho-Agronomic Characteristics of Soybean (*Glycinemax L.*), *American Journal Plant Science*, 3:331-337. <https://doi.org/10.4236/ajps.2012.33039>
- Mutant Data Base. (2017). Retrieved May 23, 2020 from Available from: <https://mvd.iaea.org/Search>
- Oladosu, Y., Rafii, M. Y., Abdullah, N., Hussin, G., Ramli, A., Rahim, H. A., Miah, G., & Usman, M. (2016). Principle and application of plant mutagenesis in crop improvement: a review. *Biotechnology & Biotechnological Equipment*, 30(1), 1-16. <https://doi.org/10.1080/13102818.2015.1087333>
- Reddy, V. R. K., & Suganthi, C. P. (1993). Effect of different ploidy levels on chlorophyll mutations frequency in some cereals. *Advances in plant sciences*, 6(1), 178-191.
- Sağel, Z., Peşkirçioğlu, H., Tutluer, M. İ. (2002). Use of Nuclear Techniques in Plant Breeding. TAEK, Ankara Nuclear Agriculture and Livestock Research Center, III. Mutation Breeding Course. 16-20 September, Ankara, Türkiye.
- Sağel, Z., Tutluer, M. İ., Peşkirçioğlu, H., Kunter, B., Kantoğlu, Y. (2013, 10-14 November). *Soybean, Tobacco, Chickpea Varieties and Characteristics Developed by Mutation Breeding*. [Conference presentation]. International Plant Breeding Congress, Antalya, Türkiye.
- Singh, V. and Nimbkar, N. (2006) Safflower (*Carthamus tinctorius L.*). In: Singh, R.J., Ed., Genetic Resources Chromosome Engineering, and Crop Improvement: Oil Crops, CRC Press, New York, 168-194.
- Solanki, R. K., Gill, R. K., Verma, P., & Singh, S. (2011). Mutation Breeding in Pulses: An overview. In: Khan, S., Kozgar, M. I. (Eds.), *Breeding of pulse crops* (pp. 85-103). Kalyani Publishers, Ludhiana.
- Tarighi, J., Mohtasebi, S. S., & Mahmoodi, A. (2010). Effect of moisture content on some physical properties of safflower (var. Darab) seeds. *Journal of Food, Agriculture & Environment*, 8(3-4), 602-606.
- Tonnemaker, K. A., Auld, D. L., Thill, D. C., Mallory-Smith, C. A., & Erickson, D. A. (1992). Development of sulfonylurea-resistant rapeseed using chemical mutagenesis. *Crop science*, 32(6), 1387-1391. <https://doi.org/10.2135/cropsci1992.0011183X003200060016x>
- Yalcin, C., & Ulakoglu, G. (2019). Determination of proper gamma radiation doses in sunflower varieties. *Int. J. Sci. & Technol. Res*, 5(9), 25-33. <https://doi.org/10.7176/JSTR/5-9-04>
- Yazıcı, L., Çiçek, S., Küçükataban, F., Çoban, M., & Tuncel, N. (2016). Determination of appropriate gamma ray dose and effect on seedling growth in M₁ of different gamma ray dose in cotton (*Gossypium hirsutum L.*) variety Nazilli 663. *Journal of Central Research Institute for Field Crops*, 25(2), 88-93. <https://doi.org/10.21566/tarbitderg.281862>