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Gamma rays and ethyl methanesulfonate applied to lentil cultivars

Abstract – The objective of this work was to evaluate the effect of gamma radiation and ethyl methanesulfonate application to four lentil cultivars (Firat-87, Koc-21, Tigris, and Sakar) under both growth chamber and field conditions. One thousand seeds from each cultivar were subjected to gamma radiation doses of 100, 200, and 300 Gy and to ethyl methanesulfonate (EMS) concentrations of 20, 40, 60, 80, and 100 mmol L^{-1}. Fifty seed from each cultivar were used to record seedling traits; and the remaining seed were sown as M1 generation in the field. For all cultivars, gamma radiation and EMS concentrations showed significant effects on seedling length, seedling fresh weight, seedling dry weight, and root dry weight. The median growth reduction varied from 278.5 to 337.3 Gy for gamma radiation, and from 49.03 to 86.029 mmol L^{-1} for EMS. In the field experiments, plant height, first pod height, number of flowers, pods, and seed per plant were investigated in the M1 generation. All gamma radiation doses, especially 300 Gy, caused a reduction in the evaluated traits in every cultivar. Regarding EMS, concentrations higher than 40 mmol L^{-1} caused a reduction in all analyzed traits in every cultivar, except in Tigris. Therefore, the Tigris lentil cultivar shows potential for mutant selection for high-yield and yield traits.

Index terms: *Lens culinaris*, EMS, lentil, gamma radiation, GR_{50}, LD_{50}.

Radiação gama e metanossulfonato de etila aplicados a cultivares de lentilha

Resumo – O objetivo deste trabalho foi avaliar os efeitos da radiação gama e da aplicação de metanossulfonato de etila a quatro cultivares de lentilha (Firat-87, Koc-21, Tigris e Sakar), em condições de câmara de crescimento e em campo. Mil sementes de cada cultivar foram submetidas a doses de radiação gama de 100, 200, e 300 Gy e a concentrações de metanossulfonato de etila (EMS) de 20, 40, 60, 80, e 100 mmol L^{-1}. Cinquenta sementes de cada cultivar foram utilizadas para registrar as características das plântulas; e as sementes restantes foram semeadas em campo como geração M1. Em todas as cultivares, a radiação gama e as concentrações do EMS apresentaram efeitos significativos sobre tamanho de plântulas, massa de matéria fresca de plântulas, massa de matéria seca de plântulas e massa de matéria seca de raízes. A redução média de crescimento variou de 278,5 a 337,3 Gy para radiação gama, e de 49,03 a 86, 029 mmol L^{-1} para EMS. Nos experimentos em campo, as características altura de planta, altura da primeira vagem, número de flores, vagens e sementes por planta foram avaliadas na geração M1. Todas as doses de radiação gama, especialmente a de 300 Gy, reduziram as características avaliadas em todas as cultivares. Quanto ao EMS, as concentrações acima de 40 mmol L^{-1} causaram redução em todas as características analisadas, em todas as cultivares, exceto na Tigris. Portanto, a cultivar de lentilha Tigris apresenta potencial para a seleção de mutantes quanto às características de alta produtividade e produção.

Termos para indexação: *Lens culinaris*, EMS, lentilha, irradiação por raios gama, GR_{50}, LD_{50}.

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Introduction

Lentil (*Lens culinaris* Medik.) is important for the diet of low-income populations in developing countries. Lentil seed contain significant amounts of proteins, carbohydrates, oils, iron, calcium, phosphorus, magnesium, vitamin A, and vitamin B. Like other legumes, lentil has the ability to fix atmospheric nitrogen (N_2) with rhizobia bacteria through symbiosis, which is advantageous for this plant growth in soils with low N_2 content (Montejano-Ramírez & Valencia-Cantero, 2024).

Lentil production and productivity in Türkiye can be low or high, due to the use of low or medium yielding cultivars that are sensitive to biotic and abiotic stresses, which are related to, respectively: insects, such as *Dolycoris baccarum* (L.) and *Piezodorus lituratus* Fabricius (Mutlu et al., 2016), *Sitona*, *Bruchus*, and aphids (Bayaa et al., 1998; Chowhan et al., 2022), as well as to diseases, including soilborne fungal pathogen complexes as *Fusarium oxysporum, Fusarium solani*, *Phoma medicaginis* var. pinodella, *Alternaria* spp., and *Fusarium* spp. (Aydın & Koç, 2008); and climate change, such as waterlogging (Wiraguna et al., 2017), terminal drought (Sarker et al., 2005), temperature, low soil fertility, and frost during flowering period in March (Bicer & Sakar, 2011). Moreover, the current narrow genetic base of improved varieties is a major limiting factor to the current production of the crop and continues to be a major obstacle to lentil breeding (Tripathi et al., 2021).

Conventional breeding methods have been successful in increasing lentil genetic diversity, despite the narrow genetic base of lentil. Conventional breeding methods, such as hybridization, require more time and labor, due to their small and self-pollinating flower structure. Therefore, increasing the genetic variability in less time can be difficult. Among breeding approaches, mutation breeding has been a cost-effective and fast-breeding method for the creation of genetic variability (Laskar et al., 2019; Jahan et al., 2024).

The plant mutation breeding has been, among several others, has proved to be one of the potential tools to increase the genetic variability of lentil crops (Roy et al., 2023). Until present, the total of 18 mutant cultivars have been developed for lentil, and most of them were created by physical mutagenesis, therefore, studies are ongoing to develop new mutant lines by chemical mutagenesis in lentil (Jahan et al., 2024).

In mutation breeding program, chemical mutagenesis is the most widely used and cheapest tool to create new alleles, but chemical mutagen dose in studies on lentil crops have not been completed yet, and studies on chemical mutagenesis are still being conducted to create new mutagenicity. In addition, the range of inducing mutation with different mutagenic doses varies, depending on the genotype used and on the targeted trait (Amin et al., 2015).

The mutagens are categorized as physical (x-rays, gamma rays, UV radiations, β-particles, neutrons), chemical (base analogues, antibiotics, alkylating agents, azides, hydroxylamine and nitrous acid) or biological agents. Lentil is responsive to both chemical and physical mutagens. Mutation in lentil cause changes in traits such as plant height, growth habit, branching and stem structure, leaf morphology, inflorescence, calyx, flower and pod characters, fertility and seed color (Jeena & Singh, 2000). Gamma rays at the dose of 15 kR can induce chlorophyll mutants (Paul et al., 2002), and it produces also dwarf mutants (Sinha, 1989), fasciated fertile mutant (Tyagi & Gupta, 1991). Among the various mutagens, EMS has been found to be more effective than salicylic acid (SA). The EMS-induced mutants vary more for growth habit and foliage types, whereas SA treatments generate more mutants for flowering behavior, maturity duration, and plant height in lentil (Khan et al., 2006). Additionally, EMS is more effective and efficient than gamma rays in inducing chlorophyll and morphological mutation in lentils (Ravi et al., 1980).

Median lethal dose (LD_{50}) and median growth reduction (GR_{50}) are utilized to establish the adequate gamma radiation dose for chemical mutagen for mutations induction. LD_{50} and GR_{50} parameters are assumptions that low doses of radiation produce minimum impacts on the genome (rarely generating phenotypic changes); whereas high doses may cause multiple impacts on the genome (deviations or negative changes), according to Álvarez-Holguín et al. (2019). Therefore, the first step in a mutagenesis-based plant breeding process is to determine LD_{50} and GR_{50}. For a successful mutation induction in plants, the dose should be below GR_{50}, in order that the size of M2 population in field and higher mutation efficiency can be easily obtained. Kangarasu et al. (2014) reported that LD_{50} dose was affected by seed size, maturity, hardiness, moisture content, and time of the treatment influences variation and cultivars of same crop.

In the present study, four different lentil cultivars were subjected to irradiation with gamma radiation at 100, 200, and 300 Gy, and to EMS concentrations at 20, 40, 60, 80, and 100 mmol L^{-1}. In growth chambers, LD_{50} and GR_{50} were determined with effective mutation doses to cultivars in the seedling period, under gamma radiation and EMS induction. In addition, in field conditions, agro-morphological traits of M1 seed, obtained after gamma irradiation and EMS mutagenic induction, were investigated.

Materials and Methods

The experiments were carried out both in plant growth chambers and in field, in the food legume area of the GAP (Southeastern Anatolia Project) International Agricultural Research and Training Center, in the 2023-2024 growing season.

Table 1. Diyarbakir meteorological data of 2023-2024 growing season, Diyarbakir, Türkiye.

Years/ months	Mean temperature (°C)	Total rainfall (mm)	Relative humidity (%)
2023			
December	12.0	140.9	73.3
November	6.9	60.3	84.4
2024			
January	5.7	87.5	82.4
February	7.4	47.8	73.5
March	9.8	101.0	69.7
April	19.0	41.4	58.2
May	20.6	42.3	53.7
June	31.0	2.5	24.4

Meteorological data of 2023-2024 growing season were obtained in Diyarbakir Meteorological Service (Turkish State Meteorological Service, 2024) and shown in Table 1. The soil type of the experiment area was reddish brown. The soil was clayey in texture, and it was very low in phosphorus and organic matter.

The lentil cultivars used in the study were Firat-87, Koc-21, Tigris, and Sakar, whose characteristics are described (Table 2). Gamma rays (Co-60) and ethyl methanesulfonate (EMS) were used as mutagen. Seed of the four cultivars were subjected to Cobalt-60 at 100, 200, and 300 Gy. For each gamma dose, 1,000 seed from each cultivar were used. Cobalt-60 radiation was performed at the Sarayköy Nuclear Research Institute of the Türkiye Atomic Energy Agency (Co-60 dose rate 0.549 kGy per hour). Untreated seed were used as controls for comparison.

In EMS ($C_3H_8O_3S$), molar mass is 124.16 g mol^{-1}, density is 1.1452 g cm^{-3} (22°C). Seed were sterilized with bleach solution for 15 min and then washed three times with water. Lentil seed were placed in a glass, and pure water was added to a volume of 1 mL per seed. Then, seeds were soaked for three hours, at 22°C, and water was decanted. The EMS solution was prepared using phosphate buffer (pH 7.0) at 20 mmol L^{-1}, 40 mmol L^{-1}, 60 mmol L^{-1}, 80 mmol L^{-1}, and 100 mmol L^{-1} (v/v) added in water. One thousand seed of each cultivar were immersed in each solution (1 mL per seed) and incubated for six hours at 20°C. EMS-treated seed were washed five times for 5 min with water. Lentil seed were washed again with running tap water for six hours. Untreated seed were used as controls for comparison.

The treated seed of gamma rays (50 seed) and EMS (50 seed) were sown in polyethylene bags containing perlite at 1:1 ratio, and placed in the plant growth chamber at 24±1ºC temperature. The experiment was

Table 2. Plant and phenological characteristics of four lentil (*Lens culinaris*) cultivars used in the study.

Characteristics	Firat-87	Tigris	Koc-21	Sakar
Plant height (cm)	40-50	21-31	33-52	24-45
First pod height (cm)	16-20	9-15	14-27	15-21
Plant growth habit	Semierect	Erect	Erect	Semierect
Number of days to flowering (days)	162-167	130-150	121	120
Number of days to maturity (days)	190-230	178	174	150
1000 seed weight (g)	35-40	25.3-37.3	31.8-49.3	39-41
Cotyledon color	Red	Red	Red	Red

carried out in a completely randomized design with three replicates. The polyethylene bags were irrigated every day. Emergence tests in bags were ended at the 14th day, and seedling length, root length, seedling fresh and dry weights, root fresh and dry weights, and number of leaves per plant were measured. Normal seedlings were weighed for fresh weight, and the seedling length and root length were measured, after which all samples were oven dried at 75°C, for 12 hours, and weighed.

For field experiment, the area was plowed in the autumn season, the seedbed was prepared by disc harrowing and bottoming. Sowing rows were furrowed manually, and 880 treated seeds were planted in the field as M1 generation.

The experiment was carried out in a randomized complete block design, with three replicates, in the 2023-2024 growing season, with three rows of 1.50 m length per plot, and 0.30 m distance between rows. A total of 880 seed were sown on December 19, 2023, distributed in 90 seed in each row, being 200 seed per square meter. M1 plants were harvested on their maturity dates as about 1 to 30 plants from each gamma radiation and EMS treatments (Table 3).

Plant height, first pod height, and number of flowers on the flower peduncle were recorded on five plants over three replicates. Number of samples for number of pods and seed per plant, and seed yield per plant were given (Table 3). Number of days to flowering and maturity, and 100-seed weight were recorded for plots on three replicates (Table 4).

After 14 days of sowing in the growth chamber, seedling length was measured, and mean growth reduction (GR_{50}) was estimated, using the resulting regression equation (y=ax+b) from the seedling length for gamma ray doses and EMS concentrations. GR_{50} was calculated using the regression equation (y=ax+b).

In gamma-irradiated seed, the median lethal dose (LD_{50}) was based on the number of seed after 30 days of sowing (DAS 30) in the field. The corresponding probit value was generated using the probit table over mortality percentage (Robertson et al., 2017), and regression was analyzed in Microsoft excel. LD_{50} was calculated using the regression equation (y=ax+b) based on the probit value.

Results obtained from both growth chamber and field experiments were analyzed using the JUMP package program, and were subjected to a variance

Table 3. Number of lentil (*Lens culinaris*) plants surviving at harvest under gamma radiation and EMS doses.

Gamma radiation dose (Gy)	Firat-87	Koc-21	Sakar	Tigris
0	15	15	15	15
100	30	24	23	25
200	30	22	22	25
300	14	16	19	25
EMS doses (mmol L^{-1})	Firat-87	Koc-21	Sakar	Tigris
0	15	15	15	15
20	15	30	30	30
40	5	20	27	29
60	-	2	7	29
80	-	-	1	25
100	-	2	1	25

Table 4. Number of days to flowering and maturity for the Firat-87, Koc-21, Sakar, and Tigris lentil (*Lens culinaris*) cultivars, for each dose of gamma radiation and ethyl methanesulfonate (EMS) treatments.

Gamma radiation dose (Gy)	Flowering (days)				Maturity (days)				100 seed weight (g)			
	Firat-87	Koc-21	Sakar	Tigris	Firat-87	Koc-21	Sakar	Tigris	Firat-87	Koc-21	Sakar	Tigris
0	110	107	106	107	166	159	159	157	4.12	4.37	4.59	3.15
100	110	108	106	107	164	161	162	159	4.33	5.06	4.72	3.42
200	109	107	107	108	169	166	166	166	4.26	4.62	5.31	3.66
300	112	109	108	109	169	166	156	167	4.14	4.88	5.52	3.91
EMS (mmol L^{-1})	Firat-87	Koc-21	Sakar	Tigris	Firat-87	Koc-21	Sakar	Tigris	Firat-87	Koc-21	Sakar	Tigris
0	111	105	106	106	170	157	157	159	4.12	4.63	4.53	3.27
20	111	105	106	106	172	167	165	167	4.22	5.43	5.26	3.58
40	111	106	106	107	-	171	171	170	-	5.49	5.38	3.79
60	111	107	107	107	-	171	173	169	-	4.75	5.24	3.75
80	112	110	109	107	-	-	-	169	-	-	-	3.66
100	113	111	110	108	-	-	-	165	-	-	-	3.66

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analysis and to the Duncan's mean comparison test, where the influence factor was the radiation dose with significance at 5% probability.

Results and Discussion

The average low temperature was in the seedling period of December, January, and February, in which plant development was slow. Temperature and precipitation increased in late March and early April, when plants were flowering. The temperature increased in May, during the seed formation period, and precipitation decreased completely towards June (Table 1).

There were differences for the effect of doses, between gamma irradiation and EMS treatments, in all cultivars, for seedling length, seedling fresh weight, seedling dry weight and root dry weight (Tables 5 and 6). Seedling length and seedling fresh and dry weight decreased at high gamma-radiation doses, in comparison with the control. The effect of gamma ray doses on root fresh and dry weight values differed among cultivars. The highest root fresh weight was in obtained in 'Sakar' at 300 Gy dose. Gamma ray doses affected the root length in 'Firat-87' and 'Koc-21', but not in 'Sakar' and 'Tigris'.

The rapid assessment for mutagenicity and identification of cultivars with low resource needs is a

Table 5. Analysis of variance and means for the phenological characteristics and median growth reduction (GR50) of seedlings of the Firat-87, Koc-21, Sakar, and Tigris lentil (*Lens culinaris*) cultivars, which were grown in chamber from seed subjected to gamma radiation doses[(1)].

Cultivar	SFW (g)	SDW (g)	RL (cm)	RFW (g)	RDW (g)	LS
Firat-87			Gamma radiation dose (Gy)			
0	0.23a	0.040a	16.6b	0.033a	0.041a	23.4
100	0.22a	0.036a	20.4a	0.032a	0.029b	22.3
200	0.20a	0.029b	20.6a	0.027a	0.024b	24.8
300	0.16b	0.027b	18.4ab	0.015b	0.013c	25.0
Dose	0.0092**	0.00037**	35.467*	0.0008**	0.00132**	16.092^{ns}
Error	0.0012	0.00003	9.156	0.00005	0.000032	13.56
Koc-21						
0	0.23a	0.038a	26.3a	0.075	0.0327a	25.6a
100	0.18b	0.025b	18.3b	0.060	0.0157c	21.8b
200	0.24a	0.037a	20.3b	0.035	0.0236b	21.2b
300	0.17b	0.027b	20.0b	0.020	0.0240b	21.1b
Dose	0.01228**	0.00043**	122.2**	0.0061^{ns}	0.00048**	45.758*
Error	0.0017	0.00001	21.064	0.003	0.000006	13.96
Sakar						
0	0.246a	0.041a	21.8	0.0357b	0.0299a	28.2a
100	0.193b	0.032b	18.1	0.0255b	0.018b	22.0b
200	0.178b	0.027c	19.1	0.0373b	0.029a	21.7b
300	0.166b	0.026c	17.3	0.0544a	0.019b	21.1b
Dose	0.01236**	0.00048**	38.42^{ns}	0.0014**	0.0004**	110.3**
Error	0.00154	0.00001	18.43	0.00024	0.00001	21.79
Tigris						
0	0.202a	0.029a	20.1	0.034	0.022a	22.1
100	0.173bc	0.024b	22.2	0.031	0.020b	20.7
200	0.187ab	0.024bc	18.7	0.043	0.022a	22.3
300	0.148c	0.022c	20.5	0.043	0.017c	19.0
Dose	0.00525**	0.000083**	20.758^{ns}	0.00041^{ns}	0.000047**	23.292^{ns}
Error	0.0009	4.815e-6	9.253	0.000184	2.295e-6	12.31

[(1)]Means followed by equal letters do not differ, by Duncan's test, at 5% probability. *,**Significant at 5% and 1% probability, respectively, by Duncan's test. ^{ns}Nonsignificant. Characteristics: SFW, seedling fresh weight (g); SDW, seedling dry weight (g); RL, root length (cm); RFW, root fresh weight (g); RDW, root dry weight (g); LS, number of leaves per seedling.

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major benefit for seedling stage screening. According to the regression equation for seedling length in the Firat-87, Koc-21, Sakar, and Tigris cultivars, the GR_{50} occurred at 337.3 Gy, 278.5 Gy, 287.1 Gy, and 314.4 Gy, respectively (Figure 1). However, higher doses were needed for 'Firat- 87' and 'Tigris'. Pramanik et al. (2023) reported a GR_{50} value of 217.2 Gy based on seedling length and root length.

There were significant differences among EMS doses for all cultivars, which showed a decrease, in comparison with the control, as EMS doses increased. Root length in 'Sakar' did not differ significantly

Table 6. Analysis of variance and means for the phenological characteristics of seedlings of the Firat-87, Koc-21, Sakar, and Tigris lentil (*Lens culinaris*) cultivars, grown in chamber from seed subjected to doses of ethyl methanesulfonate (EMS)[(1)].

Cultivar	SFW (g)	SDW (g)	RL (cm)	RFW (g)	RDW (g)	Nº of LS
			EMS(mmol L^{-1})			
Firat-87						
0	0.28a	0.047a	23.1a	0.171a	0.028a	28.7a
20	0.29a	0.049a	19.4b	0.030e	0.028a	28.8a
40	0.24b	0.038b	17.7b	0.025e	0.023b	26.6a
60	0.17c	0.023c	13.4c	0.092b	0.014c	22.3b
80	0.12d	0.018d	13.8c	0.062c	0.012c	19.9bc
100	0.08e	0.017e	7.95d	0.039d	0.007d	18.8c
Dose	0.0761**	0.0024**	283.414**	0.0307**	0.00079**	196.86**
Error	0.0009	0.00003	11.355	0.00007	0.00001	14.124
Koc-21						
0	0.29a	0.048a	18.6b	0.205a	0.034a	32.0a
20	0.23b	0.034b	21.9a	0.116b	0.026b	28.7ab
40	0.18c	0.029bc	19.2ab	0.076c	0.016c	27.6b
60	0.17c	0.027c	13.4c	0.058d	0.012d	27.3b
80	0.12d	0.018d	12.5c	0.041e	0.008e	22.4c
100	0.07e	0.011e	7.5d	0.018f	0.006e	19.3c
Dose	0.061**	0.0016**	284.93**	0.0454**	0.00117**	208.66**
Error	0.0015	0.00004	13.611	0.00016	8.655e-6	22.623
Sakar						
0	0.29a	0.051a	20.6a	0.063c	0.038a	35.3a
20	0.29a	0.049a	20.8a	0.199a	0.033b	29.2b
40	0.23b	0.036b	20.0a	0.024d	0.023c	29.2b
60	0.19c	0.028c	14.4b	0.089b	0.015d	33.3ab
80	0.01d	0.016d	11.9b	0.011e	0.010e	22.2c
100	0.09d	0.012d	6.9c	0.016e	0.005f	16.2d
Dose	0.0823**	0.0027**	325.504**	0.0514**	0.00166**	512.03**
Error	0.0014	0.000019	15.217	0.00008	9.135e-6	26.233
Tigris						
0	0.30a	0.049a	21.6a	0.211a	0.034a	30.3a
20	0.20b	0.031b	20.1ab	0.133b	0.024b	28.0b
40	0.18b	0.026c	16.6bc	0.078c	0.019c	23.8c
60	0.01c	0.014d	13.1cd	0.041d	0.010d	20.1d
80	0.07cd	0.01e	10.2de	0.024e	0.007e	16.8e
100	0.05d	0.007e	6.8e	0.029e	0.005f	13.7f
Dose	0.089**	0.0024**	330.354**	0.0537**	0.0012**	415.18**
Error	0.001018	0.000018	21.574	0.00006	4.81e-6	6.487

[(1)]Means followed by equal letters do not differ, by Duncan's test, at 5% probability. *,**Significant at 5% and 1% probability, respectively, by Duncan's test. [ns]Nonsignificant. Characteristics: SFW, seedling fresh weight; SDW, seedling dry weight; RL, root length; RFW, root fresh weight; RDW, root dry weight; LS, number of leaves per seedling.

between the control and the 20 and 40 mmol L^{-1} treatments. Conversely, in 'Koc-21', root length was significantly higher at both 20 and 40 mmol L^{-1} than control. The number of leaves per plant in 'Firat- 87' was similar to that of the control, at the doses 20 mmol L^{-1} and 40 mmol L^{-1}; however, the dose at 100 mmol L^{-1} caused a sharp decrease in all seedling traits (Table 6).

According to the regression equation for seedling length in cultivars Firat-87, Koc-21, Sakar, and Tigris, the GR_{50} occurred at 86.029 mmol L^{-1}, 64.3 mmol L^{-1}, 64.06 mmol L^{-1}, and 49.03 mmol L^{-1}, respectively (Figure 1). The first stage in mutation breeding is to identify the dose, since the radio sensitivity of every species, cultivars, and genotypes are completely different. The LD_{50} should be considered the optimal dose that causes high frequency of favorable mutation with minimum damage to the plant, since doses lower than LD_{50} favors the plant recovery after treatments, while the use of higher doses increases the possibility to induce mutations either in positive or negative ways. Therefore, to avoid an excessive loss of actual experimental materials, LD_{50} test is conducted before any mutation breeding program (Kumar et al., 2020). El-Mouhamady et al. (2020) reported that ethyl methanesulfonate (EMS) at 0.3 mmol L^{-1} dose was superior in all traits than that of the control group, and 0.3 mmol L^{-1} EMS dose showed a higher performance than 0.1 and 0.2 mmol L^{-1} EMS.

Although the GR_{50} value for lentil seed treated by gamma irradiation showed that a dose above 300 Gy was appropriate, the dose at 300 Gy was decided as the highest dose for seed in the present study. The number of seedlings per plot was accounted 30 days after sowing; and the LD_{50} values for the four lentil cultivars were determined over the number of seedlings per plot for 30 days after sowing. With the analysis of the probit curve, it was possible to show that the LD_{50} for each cultivar was different. The LD_{50} dose in 'Koc-21' (LD_{50}=127.384) and 'Sakar' (LD_{50}=131.45) was lower than 150 Gy gamma radiation. The LD_{50} dose for 'Tigris' (LD_{50}=156.75) and 'Firat-87' (LD_{50}=178.84) was higher than 150 Gy gamma radiation (Figure 2). LD_{50} dose of gamma rays in a lentil cultivar was calculated as 104.34 Gy based on the germination percentage (Tabti et al., 2018). Sharma & Sharma (1986) reported that the lethal dose for lentil types usually varied from 135 to 186 Gy. The GR_{50} and LD_{50} values are very significant, as they increase the probability of acquiring the desired mutants. Low doses of radiation and EMS treatments cause small changes in the genome. However, high doses result in unwanted or lethal mutations.

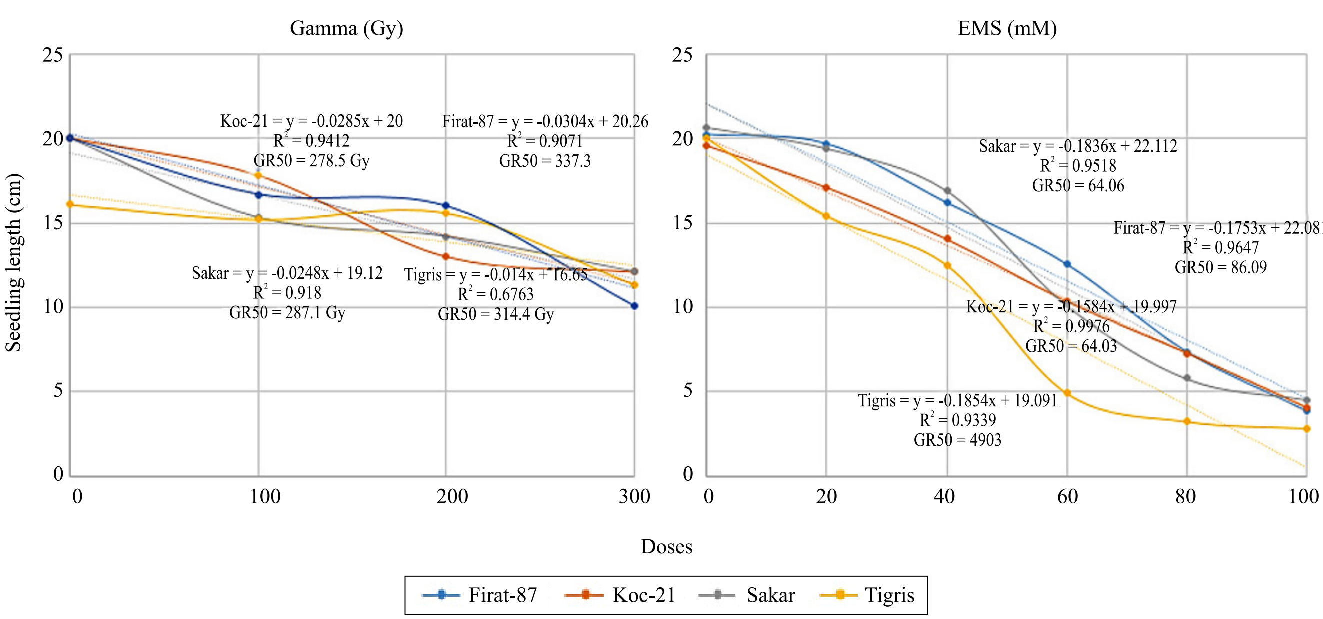

Figure 1. Median growth reduction (GR_{50}) values on the length of seedlings from seed of the Firat-87, Koc-21, Sakar, and Tigris lentil (*Lens culinaris*) cultivars (grown in chamber), subjected to gamma radiation and ethyl methanesulfonate (EMS) doses.

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In 'Firat-87', gamma radiation doses affected the plant height, first pod height, and seed yield per plant. The effect of gamma radiation doses on 'Koc-21' were significant for all traits. In 'Sakar', the doses of gamma rays affected the plant height, first pod height, and the number of flowers and seed per plant, with differences among doses. In 'Tigris', the irradiation treatment with gamma rays affected the number of pods per plant and seed yield per plant (Table 6).

The EMS doses effect showed a significant difference among all traits in 'Koc-21' and 'Sakar'. In 'Firat-87', the EMS doses affected plant height, number of flowers per peduncle, number of pods and seed, and seed yield per plant. In 'Tigris', EMS affected the first pod height, number of pods and seed per plant, and seed yield per plant (Table 6).

M1 plants were counted 30 days after planting and at harvest. While almost all seed planted at 100 Gy emerged, the number of emerged and harvested plants decreased significantly, depending on the variety at increasing radiation doses (Figure 3). Similarly, Pramanik et al. (2023) reported that the germination efficiency and germination percentage were delayed with increasing radiation doses. In Koc-21 cultivar, the number of seedlings emerging from the first dose of 100 Gy was less than 1/3, when the next dose of 200 Gy was to be applied. The number of plants harvested at 300 Gy was lower in cultivar Sakar (27.0) and in Koc-21 (32.0) than in Firat-87 (40.0) and Tigris (65.0). Laskar et al. (2018) reported that 300 Gy gamma rays in lentils could be successfully applied to a wide genetic variation through micro mutations, although it reduced the survival rate of the plants.

Although 4/3 of the planted seed germinated in field, in treatments with all EMS doses, at the harvest time surviving plants were only those of 'Firat 87', 'Koc-21', and 'Sakar' treated at 20 mmol L^{-1} and, rarely, those subjected to 40 mmol L^{-1}. The number of surviving plants increased as EMS doses was increased in 'Tigris'; in addition, at the harvest time, the highest number of 'Tigris' plants per plot were those treated at 20 mmol L^{-1} (121.0), 40 mmol L^{-1} (100.0),

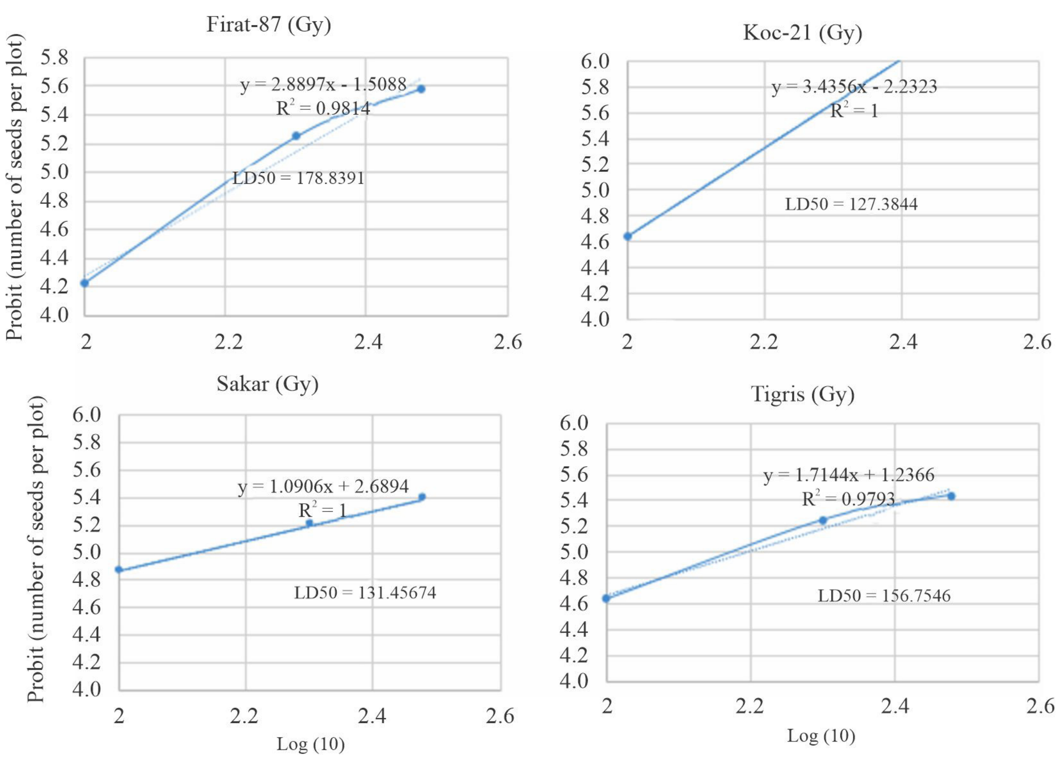

Figure 2. Lethal dose (LD_{50}) based on the number of seed that emerged 30 days after sowing (DAS 30), grown in field conditions, from seed of the Firat-87, Koc-21, Sakar, and Tigris lentil (*Lens culinaris*) cultivars, subjected to gamma radiation doses.

and 60 mmol L^{-1} (65.0). However, at seedling stage, GR_{50} values were determined at high EMS dose for 'Firat-87' (86.029 mmol L^{-1}), 'Koc-21' (64.3 mmol L^{-1}), 'Sakar' (64.06 mmol L^{-1}), and 'Tigris' (49.03 mmol L^{-1}) (Figure 3). High values of GR_{50} and LD_{50} in the seedling period caused lethal mutations, even at low doses under field conditions, due to environmental factors.

Gamma radiation doses affected plant height and first pod height in 'Firat-87', 'Koc-21' and 'Sakar', as well as the number of flowers per peduncle in 'Koc-21'. EMS doses affected plant height in 'Firat-87' and 'Koc-21', and first pod height in 'Koc-21', 'Sakar', and 'Tigris'. EMS doses affected the number of flowers per peduncle of all cultivars (Table 6).

Plant height in 'Firat-87' and 'Koc-21' (42.2 cm and 41.0 cm, respectively) was not affected by gamma radiation at 100 Gy, which did not differ from their controls (45.7 cm and 40.1 cm, respectively). In addition, for 'Sakar', 100 Gy (35.0 cm) and 200 Gy (33.3 cm) gamma radiation did not differ from the control (41.3 cm). First pod height decreased by increasing gamma radiation dose in in all cultivars, except for Tigris (Table 7).

Plant height in 'Koc-21' and 'Sakar' was not affected by EMS dose at 20 mmol L^{-1} (43.0 cm and 41.0 cm, respectively), in comparison with their controls (40.6 cm and 43.6 cm, respectively). In addition, in 'Firat-87', the effect of 20 mmol L^{-1} (46.8 cm) EMS did not differ from the control (43.2 cm). The number of flowers per peduncle decreased as EMS doses increased in all cultivars, except for 'Tigris' (Table 7). While low doses of gamma radiation caused small changes in plant height, higher radiation doses shortened this parameter. Similarly, Verma et al. (1999), Laskar & Khan (2017) and Roy et al. (2023) reported that plant height reduced with increasing concentrations of radiation doses.

The number of flowers per peduncle ranged from 2.0 to 2.4 on the control plants, in all cultivars; however, this variable in the EMS-treated plants were reduced from 1.1 to 1.9 in 'Firat-87', 'Koc-21', and 'Sakar', as a result of high dose applications. The number of flowers per stem, which is an important productivity parameter, is significantly related to the yield potential of food legumes. Laskar (2018) founded a genotype multipodding and very small seed named 'mutant

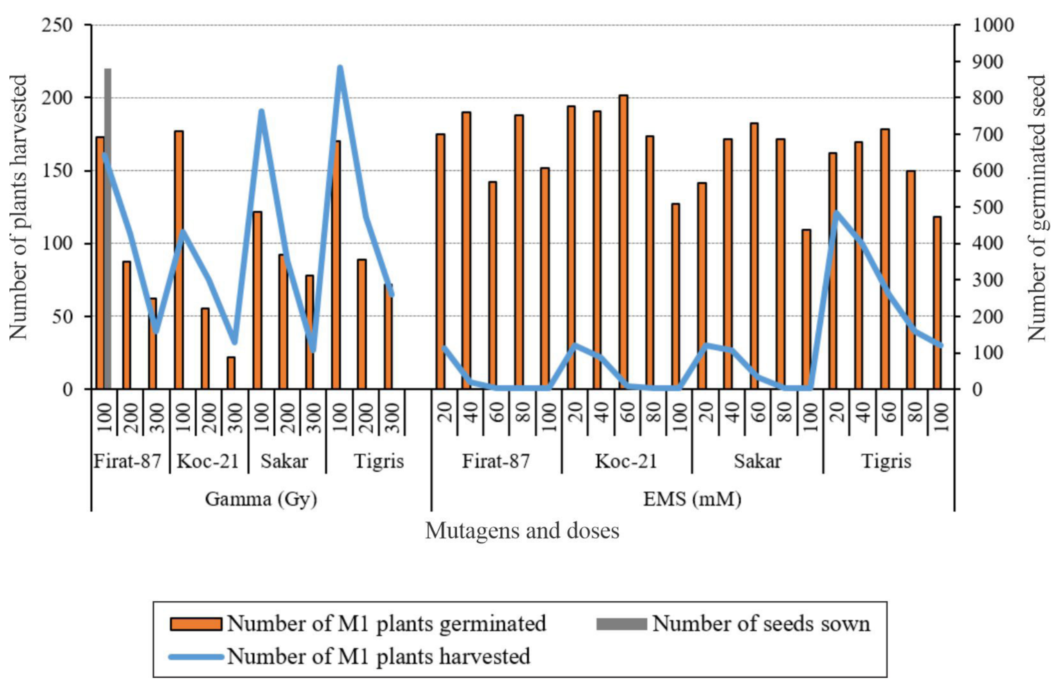

Figure 3. Number of M1 plants from seed of the Firat-87, Koc-21, Sakar, and Tigris lentil (*Lens culinaris*) cultivars (grown in field conditions), subjected to gamma radiation and ethyl methanesulfonate (EMS) doses at the stages of sown, germination and harvest.

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mp' obtained from the 'Pant L 406' cultivar, treated at 300 Gy.

The lentil cultivars showed different responses to increased gamma radiation doses for the number of flowers per plant (Figure 6). The number of flowers per plant in 'Koc-21' and 'Sakar' were similar in the treatment at 100 Gy and the control. However, the number of flowers per plant drastically decreased at 300 Gy in the treatments of the other cultivars (Figure 4).

The number of pods per plant of the control in 'Koc-21' and 'Tigris' (89.3 and 76.3, respectively) was lower under gamma radiation doses at 100 Gy (105.7 and 91.0, respectively) and 200 Gy (100.3 and 135.3, respectively). In 'Koc-21', the number of pods per plant was drastically reduced at 300 Gy (25.0), in comparison with the control (89.3) and with the doses at 100 Gy (105.7) and 200 Gy (100.3). 'Tigris' consistently bloomed and podded even at high radiation doses (Figure 4). Many researchers have also reported that, similarly to seedling height, increasing gamma radiation decreases the pollen fertility percentage (Wani et al., 2019; Pramanik et al., 2023). Kumar et al. (2016) stated that more than 50% pollen sterility was observed in pea seed irradiated with high gamma doses.

Lentil cultivars were observed as continuously blooming under gamma radiation doses in the experimental plots. Although many plants in the plots had never set pods, although flowering continuously, especially at the 300 Gy dose, the flower-to-pod transformation rate was significantly higher in 'Tigris' (Figure 4).

The number of flowers per plant in 'Koc-21', 'Sakar', and 'Tigris' increased with increasing EMS doses, in comparison with the control; however, the number of flowers per plant decreased, after the dose application

Table 7. Analysis of variance for the phenological characteristics of plants of the Firat-87, Koc-21, Sakar, and Tigris lentil (*Lens culinaris*) cultivars, grown in field conditions from seed subjected to doses of gamma radiation and ethyl methanesulfonate (EMS)[(1)].

Cultivar	PH (cm)	FPH (cm)	N° of FPL	N° of FP	N° of PP	N° of SP	SYP (g)
Firat-87				Gamma radiation (Gy)			
Dose	321.85**	218.515**	0.4^{ns}	224.3^{ns}	2372.98^{ns}	3174.57^{ns}	7.63*
Error	32.146	14.617	7.64	314.25	1275.84	1580.81	2.50
Koc-21							
Dose	116.11**	63.22*	0.889*	1497*	4184.31**	5614.75**	5.798*
Error	10.667	12.0	0.167	237.8	214.5	250.08	0.979
Sakar							
Dose	65.667*	107.417**	0.305^{ns}	1758.9**	204.97^{ns}	725.64**	0.683^{ns}
Error	18.75	4.083	0.25	153.0	255.75	88.00	0.225
Tigris							
Dose	32.67^{ns}	15.33^{ns}	0.083^{ns}	1637.46^{ns}	2456.67**	1034.67^{ns}	2.339*
Error	22.08	7.83	0.083	696.9	325.25	320.33	0.575
Firat-87				EMS (mmol L^{-1})			
Dose	1024.13**	37.439	0.836**	9464.93ns	15473.3**	20574.6**	36.07**
Error	21.88	19.32^{ns}	0.118	3603.8	973.1	1279.7	2.52
Koc-21							
Dose	745.618**	79.8**	3.485**	11037.5**	15734.1**	13463**	22.598**
Error	24.822	12.79	0.356	860.5	846.5	782.3	1.419
Sakar							
Dose	654.26^{ns}	74.53**	1.2815**	6763.6**	4129.5*	7875.9*	18.51**
Error	34.96	21.18	0.322	419.9	2028.4	2432.1	4.38
Tigris							
Dose	21.74^{ns}	37.63**	0.1417^{ns}	1863.56^{ns}	43727.3**	98757.0**	106.28**
Error	18.94	6.76	0.1441	2268.1	2803.4	6800.8	7.732

[(1)]Means followed by equal letters do not differ, by Duncan's test, at 5% probability. *,**Significant at 5% and 1% probabilities, respectively, by Duncan's test. ^{ns}Nonsignificant. Characteristics: PH, plant height; FPH, first-pod height; FPL, number of flowers per peduncle; PP, number of pods per plant; FP, number of flowers per plant; SP, number of seed per plant; SYP, seed yield per plant.

at 80 mmol L^{-1} to 'Koc-21' and 'Sakar'. 'Tigris' showed a positive response to increasing EMS doses, and the highest number of flowers per plant occurred at 100 mmol L^{-1} dose (Figure 4), when the flowering period was even prolonged at high doses.

The number of pods per plant decreased by increasing EMS doses, in comparison with the control. 'Firat-87' and 'Koc-21' treated with doses higher than 40 mmol L^{-1} did not produce pods. 'Sakar' produced pods in the treatment doses at 20 mmol L^{-1} (56.0) and 40 mmol L^{-1} (59.0), but the pod production considerably decreased at 60 mmol L^{-1} (6.9) EMS. In 'Tigris', the number of pods per plant was high at all EMS doses, in comparison with the control, and twice as many pods were produced at the highest EMS dose (100 mmol L^{-1}). Taziun et al. (2018) and Raina et al. (2022) reported an increased number of pods per plant in mutagen-treated plants. Wani et al. (2021) reported that the number of pods per plant in mutagen-treated plants was significantly different from that of the control, and the highest number of pods per plant was observed in plants with high number of flowers per plant.

The number of seed per plant and seed yield per plant of the studied cultivars were high at 100 Gy and 200 Gy (Figure 5 and 6). In 'Koc-21', the number of seed per plant and seed yield per plant was drastically reduced by the radiation dose at 300 Gy (26.7 and 1.6 g, respectively), in comparison with the control and the other doses. Laskar et al. (2019) reported that mutant plants had smaller seed, however, they produced more pods.

Data on the number of seed per plant and seed yield per plant were obtained from the control group and from plants treated with doses up to 40 mmol L^{-1} EMS. In 'Tigris', the number of seed per plant ranged from 69.5 at 20 mmol L^{-1} to 205.0 at 80 mmol L^{-1}; besides, at high EMS doses, 'Tigris' had higher seed yield than that of the control. In 'Tigris', a significant positive shift occurred in the M1 generation for the number of seed and yield per plant. However, grain yield is polygenic and complex. Therefore, Wani et al. (2021) stated that plant yield generally increased in the M2 generation

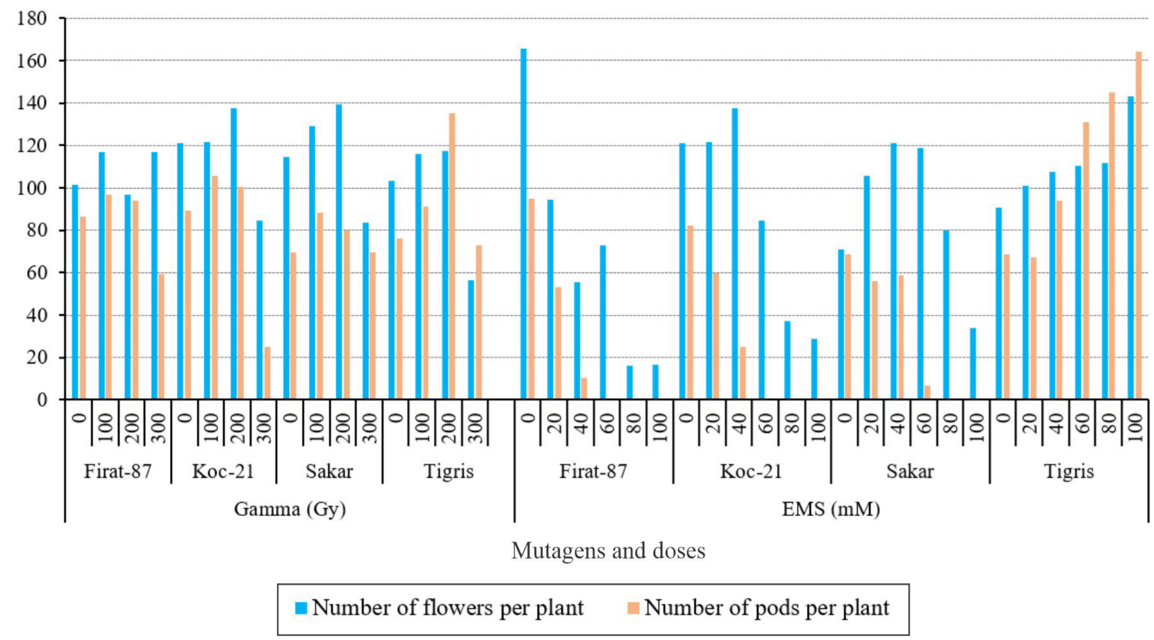

Figure 4. Number of flowers per plant and number of pods per plant from seed of the Firat-87, Koc-21, Sakar, and Tigris lentil (*Lens culinaris*) cultivars, subjected to doses of gamma radiation and ethyl methanesulfonate (EMS). Gamma radiation doses for the number of flowers per plant were significant at the probabilities of 5% in Koc-21, 1% in Sakar, and 5% in Firat-87 and Tigris. For the number of pods per plant, gamma radiation doses were significant at the probabilities 1% in Koc-21 and Tigris, and 5% in Firat-87 and Sakar. For the number of flowers per plant, EMS doses were significant at the probabilities 1% in Koc-21 and in Sakar, and at 5% in Firat-87 and Tigri'. For the number of pods per plant, EMS doses were significant at 1% probability in all cultivars.

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rather than in the M1 generation, showing a complete positive shift in the next generations. Considering the number of seed per plant and seed yield per plant, high EMS doses were lethal in 'Firat-87', 'Koc-21',

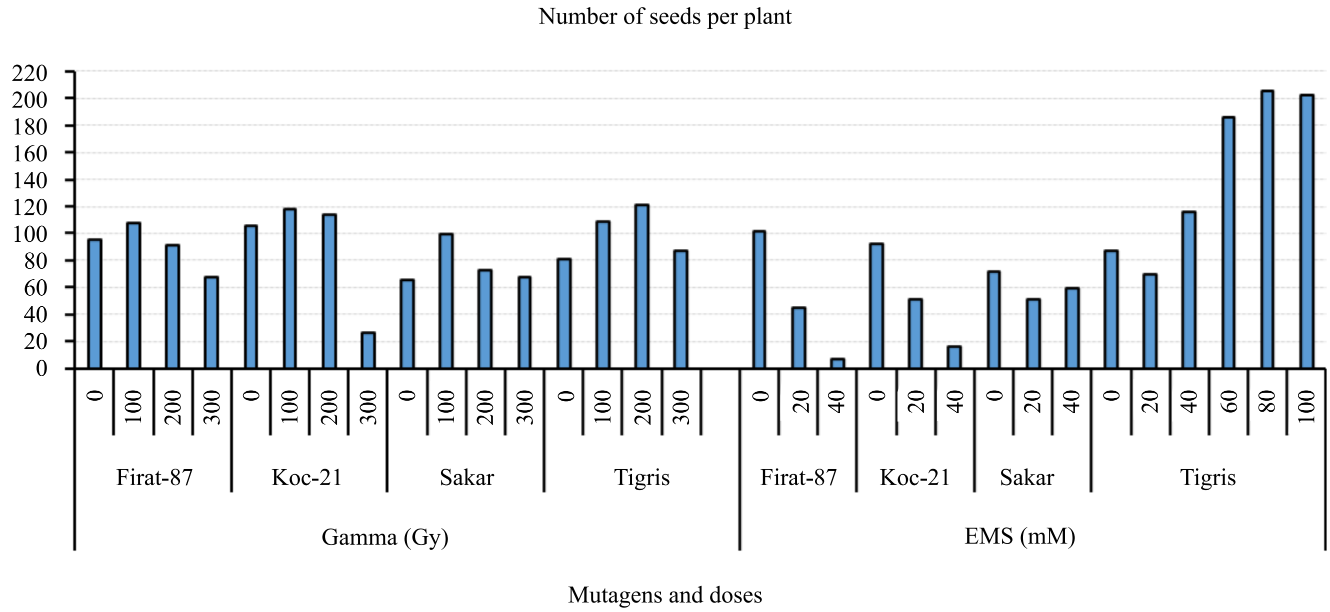

Figure 5. Number of seed per plant, from seed of the Firat-87, Koc-21, Sakar, and Tigris lentil (*Lens culinaris*) cultivars, subjected to doses of gamma radiation and ethyl methanesulfonate (EMS). Doses of gamma radiation were significant at 1% probability in Koc-21, Firat-87, and Tigris, and at 5% probability in Sakar. Doses of EMS were significant at 1% probability in Koc-21, Firat-87, and Tigris, and at 5% probability in Sakar.

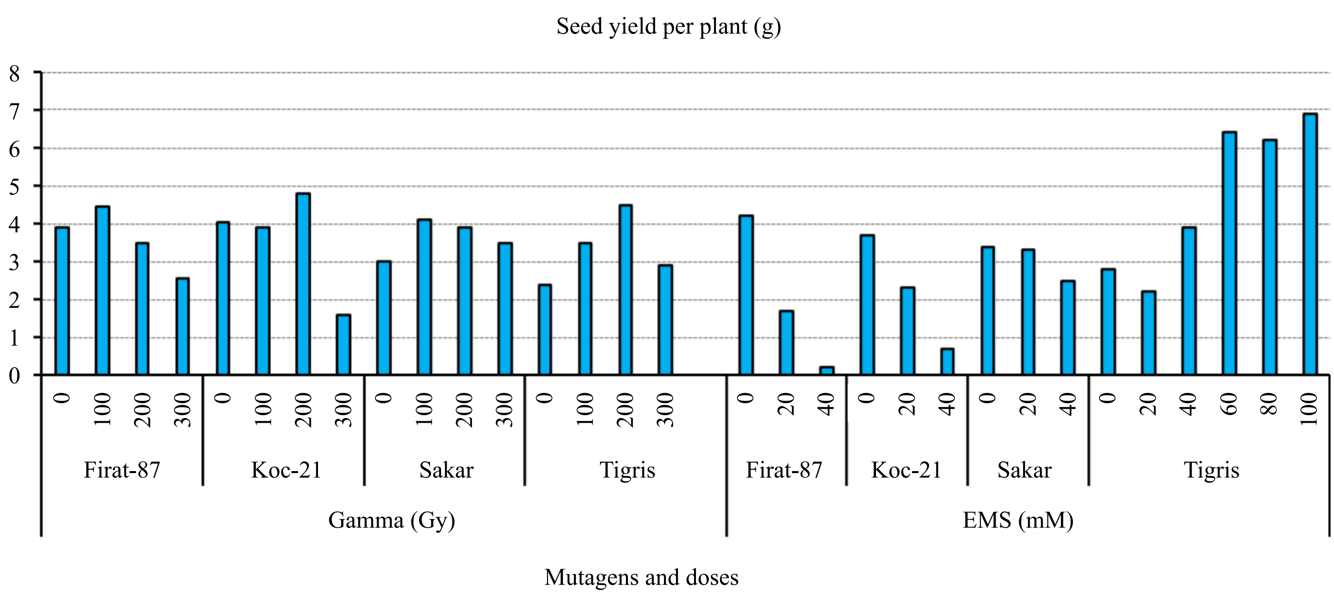

Figure 6. Seed yield per plant from seed of the Firat-87, Koc-21, Sakar, and Tigris lentil (*Lens culinaris*) cultivars, subjected to doses of gamma radiation and ethyl methanesulfonate (EMS). Gamma radiation doses were significant at 1% probability in Firat-87, Koc-21, and Tigris, and significant at 5% probability in Sakar. EMS doses were significant at 1% in all cultivars.

Table 8. Mean, standard deviation (sd), min and max, and CV percentage of plant height, first pod height, and number of flowers per peduncle of plants, from seed of the Firat-87, Koc-21, Sakar, and Tigris lentil (*Lens culinaris*) cultivars, subjected to doses of gamma radiation and concentrations of ethyl methanesulfonate (EMS)[(1)].

Cultivar	Plant height (cm)			First pod height (cm)			Number of flowers per peduncle		
	Mean±sd	Min-max	CV (%)	Mean±sd	Min-max	CV (%)	Mean±sd	Min-max	CV (%)
Firat-87				Gamma radiation (Gy)					
0	45.7±6.6a	30-55	14.5	20.6±4.6a	10-27	22.1	2.2±0.44	2-3	19.74
100	42.2±4.5ab	32-48	10.7	18.5±4.2a	12-28	22.6	2.1±0.45	2-3	19.84
200	39.1±5.3b	30-47	13.6	15.3±3.7b	8-23	24.5	2.0±0.26	1-3	13.13
300	33.2±6.6c	21-45	19.8	10.7±1.6c	7-12	14.6	1.8±0.53	1-3	28.78
Koc-21									
0	40.1±5.9a	28-51	14.8	18.0±3.8a	10-25	22.1	2.6±0.5a	2-3	20.0
100	41.0±6.6a	20-47	17.3	15.7±6.2b	9-40	43.1	2.0±0.6ab	2-4	22.6
200	33.7±6.7b	21-48	19.8	11.3±2.6bc	5-15	24.5	2.0±0.4ab	2-4	16.6
300	28.0±7.4b	12-38	29.2	7.7±3.1c	5-14	34.6	1.3±0.5b	1-2	35.6
Sakar									
0	41.3±4.3a	30-45	10.7	22.7±4.2a	15-26	21.3	2.7±0.5	2-3	20.0
100	35.0±3.4ab	30-41	9.5	13.7±2.5b	10-21	17.5	2.3±0.5	2-3	20.0
200	33.3±4.0ab	25-40	12.0	10.3±2.7bc	8-17	23.5	2.7±0.4	1-3	17.5
300	30.3±5.0b	21-39	17.3	9.7±2.0c	5-15	20.5	2.0±0.3	1-3	13.9
Tigris									
0	35.7±2.7	30-40	8.0	15.0±1.8	10-17	13.6	2.0±0.3	2-3	13.3
100	31.3±4.6	22-40	13.7	11.0±2.7	6-16	23.0	2.0±0.4	2-3	16.4
200	30.7±4.3	22-40	13.2	10.0±1.8	6-14	17.7	2.0±0.3	2-3	14.9
300	27.7±5.5	13-36	18.8	10.7±6.3	5-38	64.1	1.7±0.3	1-3	13.9
Firat-87				EMS (mmol L^{-1})					
0	43.2±4.3b	35-50	9.9	20.3±4.4	10-27	21.7	2.1±0.3a	2-3	12.5
20	46.8±4.8a	40-52	10.3	19.9±4.4	10-27	22.0	1.7±0.5a	1-2	27.7
40	39.0±5.3c	30-50	13.6	17.9±4.9	10-25	27.3	1.4±0.5b	1-2	39.1
60	34.7±4.6d	25-41	13.4	-	-	-			
80	30.2±5.1e	24-39	16.8	-	-	-			
100	24.9±3.8f	18-34	15.1	-	-	-			
Koc-21									
0	40.6±3.7a	35-45	9.1	18.4±3.9a	10-27	21.3	2.4±0.5a	2-3	21.1
20	43.0±5.0a	36-55	11.6	17.4±4.1a	10-25	23.4	1.9±0.6b	1-3	33.1
40	33.5±4.7b	25-44	13.9	14.0±2.5b	10-18	17.9	1.5±0.6c	1-3	40.1
60	34.9±4.9b	23-42	13.9	-	-	-			
80	28.8±5.8c	15-39	20.2	-	-	-			
100	24.2±5.6d	16-32	23.0	-	-	-			
Sakar									
0	43.6±5.0a	37-55	11.6	19.5±5.9a	10-36	30.2	2.3±0.5a	2-3	20.9
20	41.0±6.7ab	30-55	16.4	17.0±4.8ab	8-24	28.3	1.9±0.5ab	1-3	26.9
40	37.4±4.4b	30-45	11.7	13.8±3.0b	10-20	21.9	1.8±0.5b	1-3	37.5
60	38.0±7.2b	27-50	18.8	13.0±2b	11-20	23.7	1.1±0.5c	1-2	33.1
80	32.7±5.1c	21-42	15.7	-	-	-			
100	25.1±6.6d	10-33	26.1	-	-	-			
Tigris									
0	33.4±4.9	21-40	14.7	12.4±3.4ab	5-17	27.7	2.1±0.4	2-3	16.5
20	35.9±5.4	25-44	14.9	13.1±3.3a	9-20	24.9	2.1±0.4	1-3	19.2
40	33.0±4.1	27-42	12.4	11.6±2.2abc	9-15	18.9	2.0±0.4	1-3	20.2
60	34.7±4.5	29-44	12.9	11.3±2.7bc	7-16	24.3	2.0±0.4	1-3	20.7
80	32.8±3.0	30-40	9.2	9.0±1.6cd	5-10	18.3	2.1±0.3	2-3	14.5
100	34.0±3.7	25-40	11.0	9.7±1.7d	6-12	17.1	2.2±0.4	2-3	17.7

[(1)]Means followed by equal letters do not differ, by Duncan's test, at 5% probability.

and 'Sakar'. The lowest number of seed per plant (7.0) and seed yield per plant (0.2 g) resulted from the application of 40 mmol L^{-1} dose, and in plants of the lentil 'Firat-87' (Figure 5 and 6).

Conclusions

1. Gamma radiation and ethyl methanesulfonate (EMS) applications at increasing doses cause decreases in lentil (*Lens culinaris*) germination, seedling and root characteristics, plant height, number of flower per peduncle, number of flowers, pods, seeds and seed yield per plant.
2. In field conditions, EMS doses above 20 and 40 mmol L^{-1} concentrations show lethal effects on all cultivars, except on Tigris.
3. Among the studied cultivars, Tigris stands out due to positive mutations up to 80 mmol L^{-1} EMS for number of flower per plant, pods, seed and seed yield per plant, meaning it is promising for the development of new mutant varieties with a high yield.

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