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# DETERMINATION OF THE EFFECTS OF DIFFERENT EMS DOSES APPLIED TO SEEDS OF CHICKPEA AND LENTIL VARIETIES ON SOME SEEDLING CHARACTERISTICS


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**Abstract:** In this study, it was aimed to determine the effects of 11 different doses of Ethyl Methane Sulphonate (EMS) applied to the seed to create variation in the M1 generation of chickpea (Gökçe) and lentil (Şakar) genotypes during germination and seedling development periods and to determine the lethal dose that caused a 50% reduction in plant emergence rate. The research was conducted under the greenhouse and laboratory conditions of the Faculty of Agriculture of Dicle University in 2019/2020. The study was conducted according to a randomized block design with three replicates. For the M1 generation, seeds of each genotype in the elite stage were treated with EMS solution at 0 (control), 10, 20, 30, 40, 40, 50, 60, 60, 70, 70, 80, 90, and 100 mM (1000 seeds for each dose) and then sown in the greenhouse. A total of 132 tubes were sown with 30 seeds for each dose, and the effective EMS dose was determined for each genotype based on the traits examined in the developing seedlings. In the study, it was concluded that 11 different EMS doses applied to the seeds of chickpea and lentil varieties had negative effects on seedling development in the M1 generation, and increasing EMS doses from the control caused a decrease in all traits examined. With increasing EMS doses, plant emergence was observed in both chickpea and lentil up to 60 mM dose, while no germination was observed at 60 mM dose, and the dose rate varied according to species and varieties. The dose that caused a 50% decrease in the plant emergence rate in Gökçe chickpea and Şakar lentil varieties was determined as the LD50 dose. Accordingly, it was determined that the LD50 dose was 30 mM for Gökçe chickpea variety and 60 mM for Şakar lentil variety.



**Keywords:** Chickpea, EMS, Lentil, LD50, Mutation



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## 1. Introduction

Legumes, which have been cultivated since ancient times, are of great importance in terms of meeting the protein requirements of plant origin for human nutrition. Grain legumes, which contain high levels of crude protein, are especially rich in essential amino acids, such as Lysine, Leucine, Isoleucine, vitamins A and B, and mineral substances (Şehirali, 1988). Legumes enrich the soil in terms of nitrogen by binding the free nitrogen in the air with Rhizobium bacteria, forming nodosity in their roots (Özdemir, 2002). After the harvest of the plant, the high amount of nitrogenous organic compounds contained in the roots is decomposed by microorganisms in the soil, some of which decompose, and the plants planted later benefit from this nitrogen. In addition, legume roots aerate the soil, prevent soil compaction, improve the physical, chemical, and biological properties of the soil, and contribute to the maintenance of soil fertility (Şehirali, 1988). For these reasons, the cultivation of grain legumes in crop rotation in our country is of particular importance.

As with other crops, it is necessary to develop new varieties of legumes that are resistant to diseases and pests, suitable for machine harvesting, high quality, high yield, and suitable for the demands of domestic consumers and foreign markets. For this purpose, in conditions where genetic problems cannot be solved when developing new varieties with traditional plant breeding methods, one or two characteristics of a productive variety with high adaptability can be increased by mutation breeding. Mutations occur in two ways: spontaneous (natural) and induced (artificial). Artificial mutations are caused by physical mutagenesis (X-rays, gamma rays, ultraviolet radiation, beta radiation, neutrons), chemical mutagenesis (basic analogs and related compounds, antibiotics, alkylated substances, azides) (Spencer-Lopes et al., 2018). Induced mutation or mutagenesis is defined as sudden heritable changes in the genome of an organism that do not result from genetic recombination but are induced by physical, chemical, or biological agents (Roychowdhury and Tah, 2013). Chemical mutagens primarily induce single point mutations and contribute to the development of new varieties with improved traits, such as high yield, short plant height, and disease resistance in breeding programs (Khursheed et al., 2015; Tantray et al., 2017). Among the chemical mutagens, Ethyl Methane Sulphonate (EMS) is the most popular alkylating agent among plant breeders because it is easily detoxified and used (Pathirana, 2011; Hassan et al., 2021). EMS mutagenesis causes alkylation, so the original base is not physically altered (Kantoğlu and Kunter, 2021).

Mutation breeding studies aim to obtain the highest mutation frequency with the least damage. Mutagen doses and application methods should be selected appropriately for this purpose, and changes in M1 plants and the resulting physiological damage should be determined quantitatively. Generally, doses that kill 50-70% of the seedlings are determined as the appropriate mutagen dose and are called the LD50 dose (Lethal Dose) (Şehirali and Özgen, 1988). According to IAEA (International Atomic Energy Agency) data for 2021, 2652 varieties were registered in physical mutagen applications, 677 varieties in chemical mutagen applications, and 36 varieties in chemical + physical mutagen applications. Worldwide, 1648 mutant varieties have been registered in 15 cereal species and 424 mutant varieties in 18 legume species. In legume species, 27 chickpea (*Cicer arientum* L.) and 18 lentils (*Lens culinaris* L.) mutant varieties have been registered (IAEA, 2021).

In this study, we aimed to determine the effects of 11 different EMS doses applied to the seeds of chickpea and lentil varieties on seedling development in the M1 generation and to determine the appropriate EMS dose.

## 2. Materials and Methods

The research was carried out under the greenhouse and laboratory conditions of the Faculty of Agriculture of Dicle University in 2019/2020. In this study, eleven different EMS doses, one lentil (Şakar), and one chickpea (Gökçe) variety were used as plant materials. The study was set up in a randomized block design, with three replicates in a greenhouse.

### 2.1. EMS (Ethyl Methane Sulphonate) Application

EMS application was carried out under the laboratory conditions of the Dicle University Faculty of Agriculture. The EMS amounts calculated for different EMS doses used in this study are listed in Table 1.

EMS was applied to the seeds, according to the method described below. For each dose, 1000 seeds of each variety were first kept in pure water for 6 h, thereby increasing the permeability of the seed coat. The seeds were then thoroughly filtered and kept in 11 different EMS solutions (0 (control), 10, 20, 30, 40, 50, 60, 70, 80, 90, and 100 mM) for 6 h. The seeds were mixed every hour with a metal spoon for better mutagen penetration. The seeds were rinsed thrice with distilled water to remove EMS mutagens. The washed seeds were dried in the laboratory under air circulation. The dried seeds were planted under greenhouse conditions.

To determine the seedling growth properties of the different plant species, M1 seeds were sown in a controlled greenhouse. Peat (2/3) and perlite (1/3) were used as the soil in the greenhouse, and the seeds were sown on 24.12.2019 according to a randomized plot design with three replications. Thirty seeds were sown per EMS dose. Temperature and humidity values during seedling growth are shown in Figure 1.

The following parameters were examined: plant emergence rate (%), first leaf length (cm), root length (cm), seedling height (cm), fresh seedling weight (g), and dry seedling weight (g), which were determined by measuring the seedlings on the 28$^{th}$ day of M1 plants.

Statistical analyses of the parameter values obtained from the greenhouse experiment were performed using the JMP Pro 13 statistical package program.

**Table 1.** EMS doses used in the study

| EMS doses | Amount of EMS used (ml) | Amount of pure water used (ml) |
|---|---|---|
| Control | 0.000 | 500.000 |
| 10 mM | 0.542 | 499.458 |
| 20 mM | 1.084 | 498.916 |
| 30 mM | 1.626 | 498.374 |
| 40 mM | 2.168 | 497.432 |
| 50 mM | 2.710 | 497.290 |
| 60 mM | 3.252 | 496.748 |
| 70 mM | 3.794 | 496.206 |
| 80 mM | 4.336 | 495.664 |
| 90 mM | 4.879 | 495.121 |
| 100 mM | 5.421 | 494.579 |

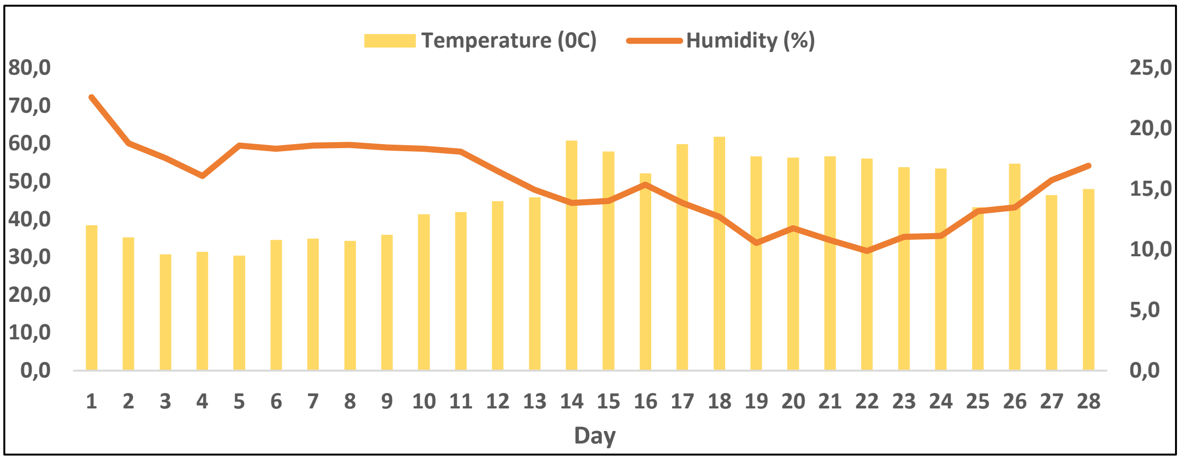


**Figure 1.** 28-day temperature and humidity data of greenhouse conditions during seedling growth of M1 legume plants.

## 3. Results and Discussion

The plant emergence rate, root length, seedling height, seedling fresh weight, and seedling dry weight were evaluated in M1 stage legume species generated with eleven different EMS doses under greenhouse conditions. ANOVA revealed that different EMS doses resulted in changes in all properties evaluated across legume species. Regarding the average seedling dry weight, no statistical differences were identified between chickpea cultivars (Figure 2, 3, 4, 5, and 6).

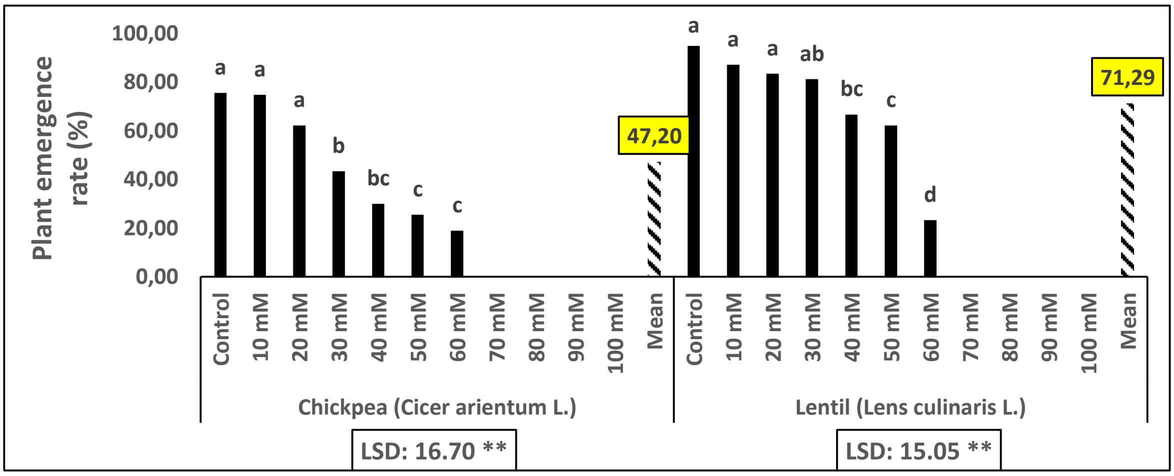


**Figure 2.** Plant emergence rates (%) of different EMS doses in chickpea and lentil. **Significant at P≤0.01, ns= not significant, LSD= least significant difference.

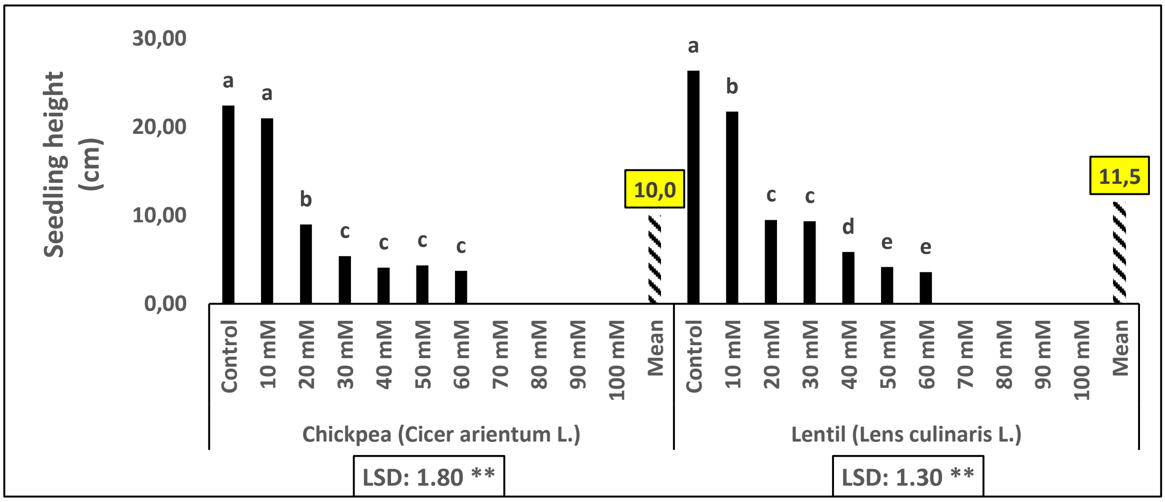


**Figure 3.** Seedling height (cm) of different EMS doses in chickpea and lentil. **Significant at P≤0.01, ns= not significant, LSD= least significant difference.

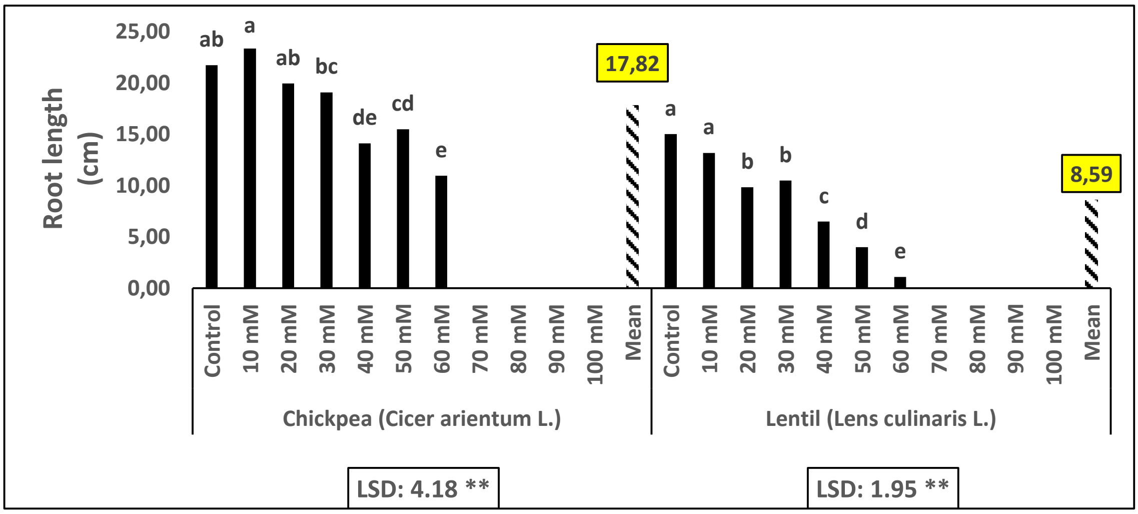


**Figure 4.** Root lenght (cm) of different EMS doses in chickpea and lentil. **Significant at P≤0.01, ns= not significant, LSD= least significant difference.

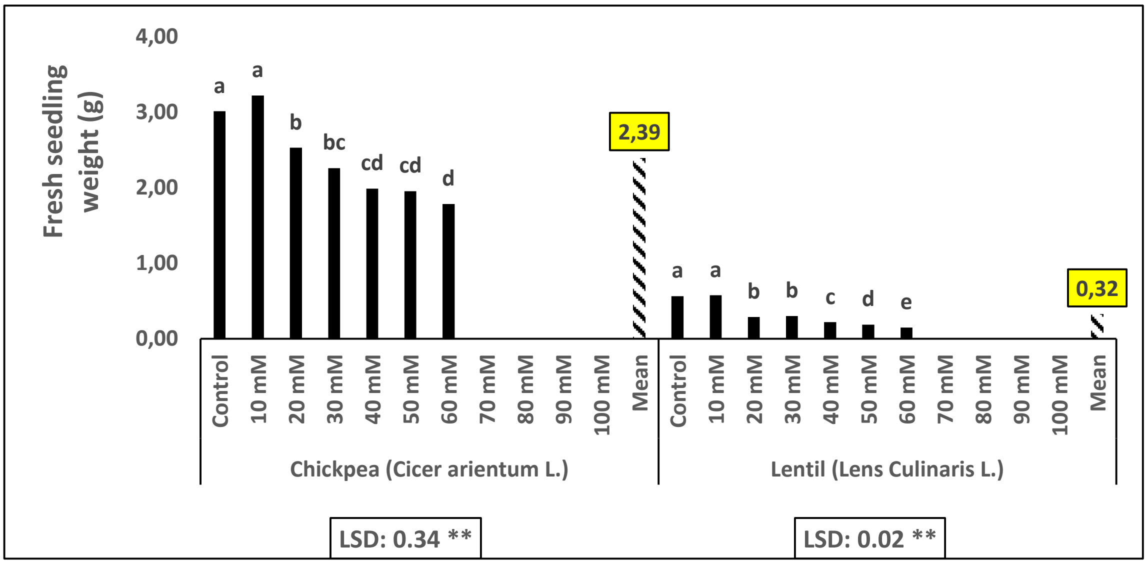


**Figure 5.** Fresh seedling weight (g) of different EMS doses in chickpea and lentil. **Significant at P≤0.01, ns= not significant, LSD= least significant difference.

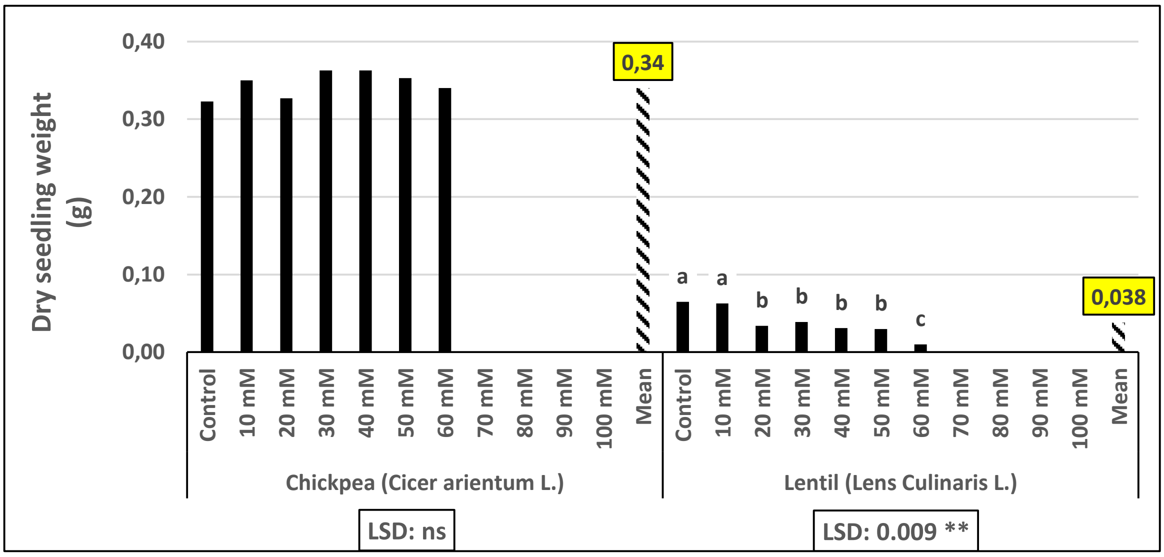


**Figure 6.** Dry seedling weight (g) of different EMS doses in chickpea and lentil. **Significant at P≤0.01, ns= not significant, LSD= least significant difference.

As shown in Figure 2, while plant emergence was observed up to a 60 mM dose in both chickpea and lentil plant species with increasing EMS doses, no germination was observed at doses of 60 mM. The plant emergence rate decreased with an increase in EMS doses in the Gökçe chickpea and Şakar lentil cultivars. The average emergence rate varied between 18.89% (60 mM EMS dose) and 75.56% (control dose) in the Gökçe chickpea cultivar and between 23.33% (60 mM EMS dose) and 94.97% (control dose) in the Şakar lentil cultivar. A higher plant emergence rate was observed in the lentils than in the chickpeas.

It was concluded that EMS and other mutation sources used as mutagens had different effects on the characteristics examined in M1 plants and that the mutagenic effect increased with an increase in dose (Talebi et al., 2012; Güvercin et al., 2020; Aher and Koche, 2022). Researchers have reported that the success of mutations depends on the efficacy of the mutagen used, duration, frequency, and genotype (Sikora et al., 2011; Anbarasan et al., 2013; Arisha et al., 2015).

Deepika et al. (2016) reported that the reduction in seed germination may be due to the effect of mutagen on the meristematic tissues of the radical/plumula. Kulkarni (2011) reported that one of the physiological effects caused by chemical mutagen application is disorders in the formation of enzymes involved in the germination process. Researchers have found that the highest emergence was observed in the control group in proportion to the increasing EMS doses in the calculation based on days from emergence in M1 plants, and the values obtained decreased significantly compared with the control (Jadhav et al., 2012; Anbarasan et al., 2013; Jagajanantham et al., 2013; Baghery et al., 2016). Similar inhibitory effect of various mutagenic treatments on seed germination has been previously reported in Lentil (Kumar and Sinha, 2003), Cowpea (Gaur et al., 2003), Chickpea (Khan and Wani, 2005; Aher and Koche; 2022), Grass Cowpea (Ramezani and More, 2013) and Cluster bean (Deepika et al., 2016).

Researchers have reported that plant emergence rate in mutagen applications in M1 generation; Akıncı (1999) 16.67 - 98.90% (300 Gy-control), Acar (2010) 38.13 - 76.63% (0.4% EMS-control), Baghery et al. (2016) 55.67 - 97.67% (1,050% EMS-control), Olaolorun et al. (2019), 71.26 - 75.92% (EMS application-control), Güvercin et al. (2020) 47.11 - 89.87% (1% EMS-control), Yorulmaz et al. (2021) 0.00 - 93.33% (30 mM EMS-control) and Aher and Koche (2022) 31.56 - 93.37% (0.3% EMS-control).

The LD50 is used by most researchers to determine the lethal dose of mutagens (Warghat et al., 2011; Talebi et al., 2012; Anbarasan et al., 2013). In every mutation-breeding program, the LD50 was initially determined, which was used as the optimum concentration for induction. If this step is ignored, the mutagen dose may be high or low and mutation frequency may occur. LD50, which is defined as the mutagen dosage resulting in a 50% reduction in seed germination after exposure of seeds to mutagen for a certain period of time and under certain conditions, is often used to compare the effect of mutagens in seeds treated under different conditions (Bharathi et al., 2013; Beyaz et al., 2016). In the study, the dose that caused a 50% decrease in plant emergence rate among 11 different EMS dose applications applied in legume species was determined as LD50 dose. Accordingly, the LD50 dose was determined to be 30 mM for Gökçe chickpea variety and 60 mM for Şakar lentil variety (Figure 2.). Özkan et al. (2021) found that increasing the dose of EMS in chickpea plants decreased the germination rate of seeds, seedling characteristics and the proportion of living healthy plants and that the effective mutation dose on all traits can be obtained from doses between 50-60 mM.

Regarding the seedling characteristics examined in this study, the highest values obtained from control and 10 mM EMS doses for chickpeas, and the control group for lentils. In addition, the highest seedling height in lentil was followed by 10 mM EMS doses. The values for these characteristics decreased proportionally with increasing EMS doses. There were variations in the intermediate doses, despite the fact that the doses at which the highest and lowest readings were attained were the same. At the end of the 28th day, the root length and seedling height of the Gökçe chickpea variety varied between 10.97 - 23.37 cm and 3.73 - 22.43 cm, respectively (Figure 3 and 4). It was determined that seedling fresh weight value varied between 1.78 - 3.22 g and the seedling dry weight value varied between 0.323 - 0.363 g between doses (Figure 5 and 4).

At the end of the 28th day, the root length and seedling height of the Şakar lentil variety varied between 1.10 - 15.03 cm and 3.60 - 26.38 cm respectively (Figure 3 and 4). The seedling fresh weight ranged from 0.147 - 0.573 g, and the seedling dry weight ranged from 0.010 - 0.065 g between the doses (Figure 5 and 4).

Researchers have reported that all traits examined during the seedling stage decrease proportionally with increasing EMS doses, and there are statistically significant differences in the traits examined during the seedling stage (Talebi et al., 2012; Lukanda et al., 2013; Özkan et al., 2021). Seedling height is often used as an index to determine the biological effects of different physical and chemical mutagens on M1 (Bhat et al., 2007). There is a linear dependence between seedling height and dose of physical or chemical mutagens (Talebi et al., 2012). In studies conducted in different plant species, researchers have shown that seedling height decreases owing to the mutagenic effect of EMS (Talebi et al., 2012; Anbarasan et al., 2013; Ambli and Mullainathan, 2014). Researchers reported that the length value measured at seedling stage varied between 8.40 - 33.04 cm (Talebi et al., 2012), 1.27 - 18.04 cm (Atmaca et al., 2012), 25.93 - 33.73 cm (Olaolorun et al., 2019) and 21.87 - 26.83 cm (Yorulmaz et al., 2021) in mutagen treatments. In agreement with these results, our findings indicated that the decrease in seedling height was caused by an increase in EMS concentration.

The highest root length value of M1 plants was generally obtained from the control group, and the values decreased with increasing doses of EMS (Shah et al., 2012; Ambli and Mullainathan, 2014). Dhakshinamoorthy et al. (2010) reported that 4% EMS treatment caused a 35% reduction in root length compared to 1% EMS treatment, while Anbarasan et al. (2013) reported that 1.8% EMS treatment reduced root length by 46% compared to 0.4% EMS treatment. Talebi et al. (2012) reported that root length decreased with increasing EMS concentration. The values of root length in mutagen treatments were reported to vary between 7.24 - 15.71 cm (Atmaca et al., 2012), 2.23 - 9.66 cm (Talebi et al., 2012) and 5.28 - 7.69 cm (Ambli and Mullainathan, 2014).

In previous studies, it was observed that seedling fresh weight and dry weight values decreased, especially in plants exposed to mutations (Atmaca et al., 2012; Yorulmaz et al., 2021). Saba and Mirza (2002) stated that there was a decrease in fruit weight compared to the control group plants with EMS application at different times and doses. Seedling fresh weight in the M1 generation has been reported to vary between 0.000 - 0.250 g (Başer et al., 2005), 0.010 - 0.630 g (Atmaca et al., 2012) and 0.850 - 0.647 g (Yorulmaz et al., 2021). The values of seedling dry weight in the M1 generation have been reported to vary between 0.025 - 0.037 g (Akıncı, 1999) and 0.071 - 0.051 g (Yorulmaz et al., 2021).

The correlation relationships of the traits measured during the seedling stage in chickpea and lentil varieties treated with 11 different EMS doses are given in Figure 7 and 8. Based on these results, it was determined that there was a significant and positive correlation between all traits examined in both the Gökçe chickpea and Şakar lentil varieties. However, there was a negative and insignificant correlation between seedling dry weight and the other traits examined in the Gökçe chickpea variety. In previous mutation applications, Başer et al. (2005) reported significant and positive correlations between emergence percentage, seedling height, seedling fresh weight, and root length traits measured at the seedling stage. Adebisi et al. (2010) observed a positive relationship between germination rate and other seedling traits measured in mutation treatment. Olaolorun et al. (2019) reported that there was a negative correlation between plant emergence rate, seedling height, and root length because seedlings with early emergence had a better root structure and a higher chance of developing into taller plants.

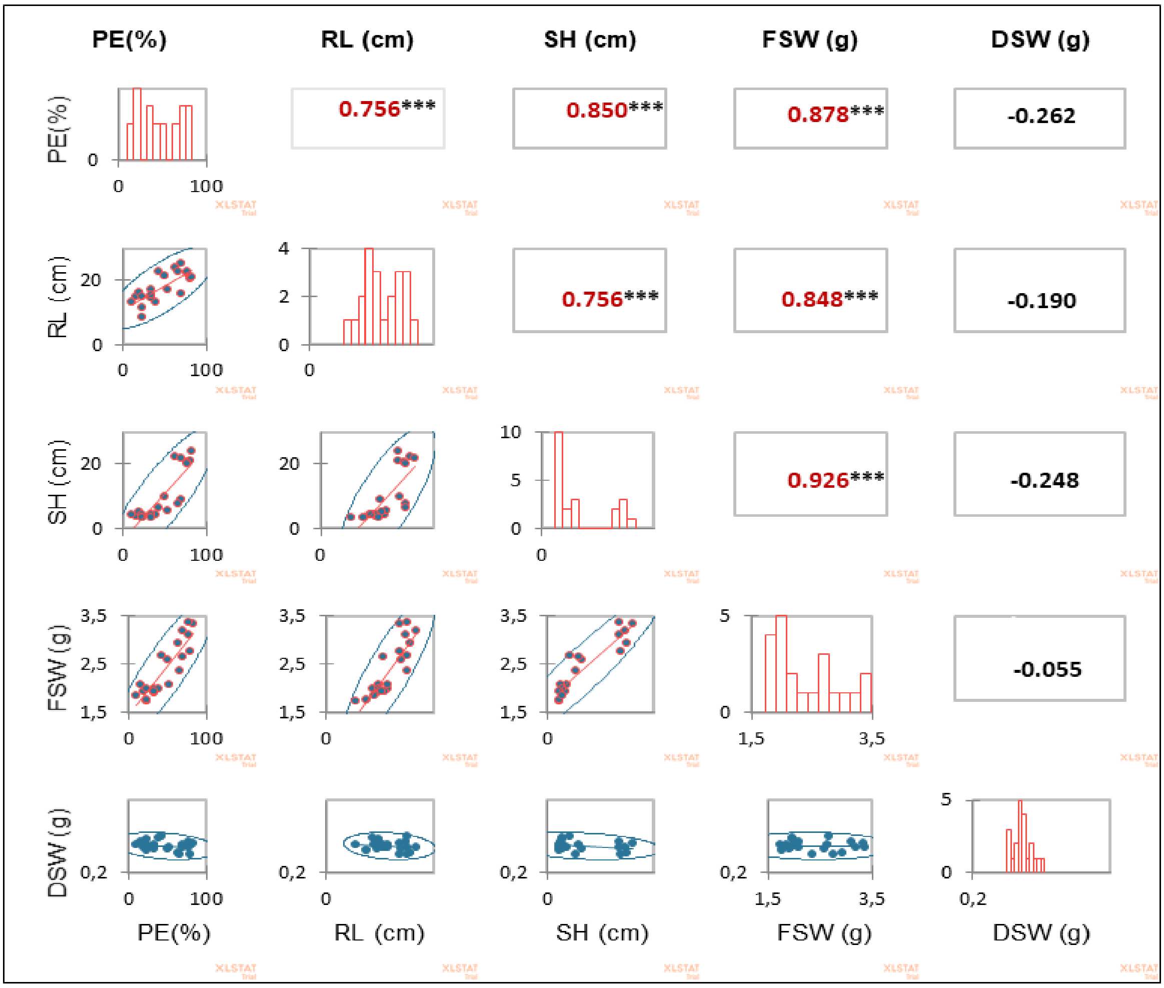


**Figure 7.** Correlation analysis of the traits analyzed in Gökçe chickpea variety. PE= plant emergence rate, RL= root length, SH= seedling height, FSW= fresh seedling weight, DSW= dry seedling weight.

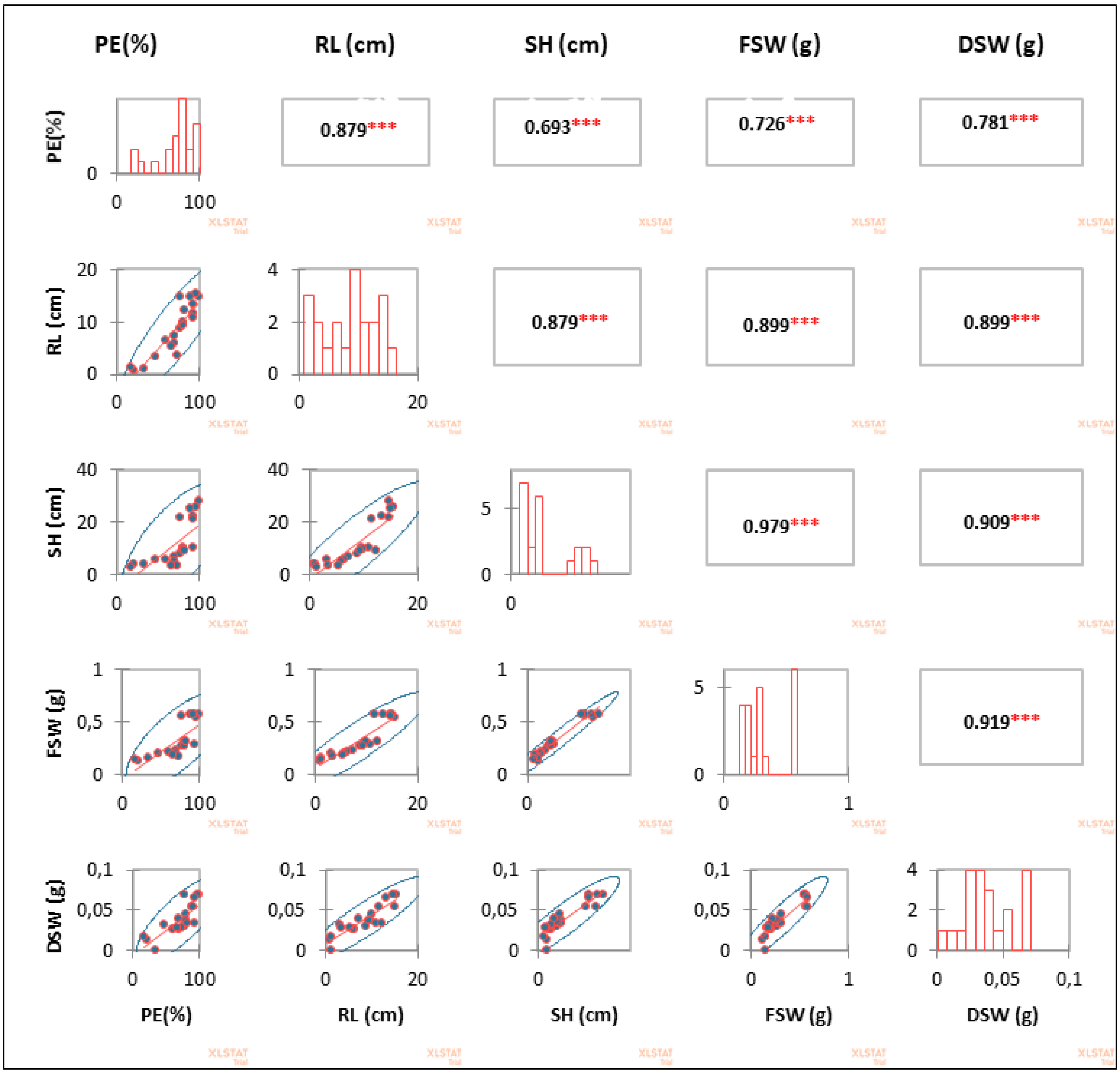


**Figure 8.** Correlation analysis of the traits analyzed in Şakar lentil variety. PE= plant emergence rate, RL= root length, SH= seedling height, FSW= fresh seedling weight, DSW= dry seedling weight.

## 5. Conclusion

This Mutation breeding is the easiest and fastest source of variation for plant breeders, and has been widely used worldwide for research purposes. From the past to the present, it has been demonstrated that artificial mutation studies can lead to positive improvements in terms of yield and yield elements, although different plant species may suffer different physiological and chemical damages in different environments and applications. Mutant varieties have been developed and registered in many plants worldwide. In this study, it was concluded that 11 different EMS doses applied to the seeds of some chickpea and lentil varieties had negative effects on seedling development in M1 generation and increasing EMS doses from the control caused a decrease in all traits examined. With increasing doses of EMS, plant emergence was observed in both chickpea and lentil up to 60 mM dose, while no germination was observed at 60 mM dose and the dose ratio varied according to species and varieties. In the Gökçe chickpea and Şakar lentil varieties, the dose that caused a 50% decrease in the plant emergence rate was determined as the LD50 dose. Accordingly, LD50 dose was determined to be 30 mM for Gökçe chickpea variety and 60 mM for Şakar lentil variety.

**Author Contributions**

The percentage of the author contributions is presented below. All authors reviewed and approved the final version of the manuscript.

| | M.B. |
|---|---|
| C | 100 |
| D | 100 |
| S | 100 |
| DCP | 100 |
| DAI | 100 |
| L | 100 |
| W | 100 |
| CR | 100 |
| SR | 100 |
| PM | 100 |
| FA | 100 |

C=Concept, D= design, S= supervision, DCP= data collection and/or processing, DAI= data analysis and/or interpretation, L= literature search, W= writing, CR= critical review, SR= submission and revision, PM= project management, FA= funding acquisition.

**Conflict of Interest**

The author declared that there is no conflict of interest.

**Ethical Consideration**

Ethics committee approval was not required for this study because there was no study on animals or humans.

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