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Determination the effective dose of mutation in pepper (*Capsicum annum* L.)

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Abstract

This study was carried out to determine the "Effective Dose of Mutation" (EMD_{50}) for mutation breeding study on Çermik, Kandil and Üçburun pepper varieties grown intensively in Diyarbakır. For this purpose, pepper seeds were exposed to chemical mutagen source Ethyl Methane Sulfonate (EMS) at doses of 0%, 01%, 0.2%, 0.3%, 0.4%, 0.5%, 0.75% and 1% and to irradiation with physical mutagen source Co^{60} at doses of 0, 50, 100, 200, 300, 400, 500 and 600 Gy. In irradiated and chemically exposed seeds, on the 45th day following seed sowing date, germination percentage (%) for each dose was calculated and seedlings' lengths were measured to investigate the effects of different doses and EMD_{50} value was calculated. According to the data obtained as a result of the study; it was found that the germination rate in pepper seeds of all three varieties-genotypes gradually decreased with the increases of doses in application of EMS on pepper seeds. While the decreases were especially evident in the seeds of Kandil and Üçburun varieties subjected to 0.3% EMS dose and 9 hours of application; in Çermik genotype the germination rate started to decrease with 0.5% EMS dose due to local population. It was also found that germinations decreased in all three varieties depending the application of 300 Gy CO^{60}. In 6-hour EMS application, EMD_{50} values were respectively determined as 0.67%, 0.97% and 1.08%. for Kandil, Üçburun and Çermik. And for 9-hour EMS application, EMD_{50} values were determined as; 0.52%, 0.77%, 0.89%. Kandil, Üçburun and Çermik varieties. According to the results obtained in the study, the usage of both physical and chemical mutagens in pepper varieties; increasing of doses and durations of mutagens, decreased germination rates and also caused to decrease the plant heights. As a result, it seems possible to obtain variations in peppers with the usage of physical and chemical mutagens to get new varieties in plant breeding.

Keywords: Pepper, Effective Mutation Dose, Gamma, Ethyl Methane Sulfonate, Germination

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INTRODUCTION

Pepper (*Capsicum* sp.), is an important species of the *Solanaceae* family that has an important position among other vegetables in terms of production area and production amount in the world and in Türkiye. According to 2022 data from the Food and Agriculture Organization of the United Nations (FAO), 36,972,410 tons of pepper produced in the area of 2,020,816 hectares in the world. According to these data, an average yield of pepper is 1,83 kg/ m2 (FAO, 2022). The countries those have the most pepper production in the world are respectively; China is first with 16,837,405 tons, Mexico is the second with 3,113,244 tons, Indonesia is third with 3,020,262 tons, Türkiye is fourth with 3,018,775 tons and Spain is fifth with 1,533,280 tons. According to these results, China alone produces half of the world's pepper production.

When we look at the pepper varieties produced in Türkiye, capia was the most grown and produced pepper in 2023 (Table 1). According to table 1, it was determined that the average yield of capia pepper is 4,27 tons/decare the long pepper is 3,73 tons/decare, bell pepper is 3,46 tons/decare and Charleston pepper is 6,78 tons/decare in Türkiye (TUİK, 2023).

Table 1. The pepper varieties grown in Türkiye

Varieties	Growing Area (Decare)	Production (Tonnes)
Capia (Paste)	375,147	1,602,457
Long	251,672	939,178
Green Bell	114,197	395,441
Charleston	21,218	143,934

Anonymous: TUIK, 2023

While to show the ability of obtaining high yields from a unit area as mathematically; "Yield = Plant's Genetic Potential + Cultivation Technique Package + Environmental Conditions" can be formulated (Kurt, 2015). It is an expected result that the high yield obtained from a unit area due to the seed containing high yield that is the source of plant production. Therefore, the seed will be used it is expected to strong in terms of desired features (quality, market value, high yield, resistance to diseases and pests, etc.).

When the seeds used in commercial production in the world and in Türkiye were investigated, it was seen that the majority of the seeds used were F_1 hybrid seeds (Tepe et al., 2003; Kantoğlu, 2014a). F_1 hybrid seeds are obtained as a result of crossbreeding between parents with superior characters that are not related in terms of genetic structure. Hybrid seeds obtained by hybridization (Heterosis) can have very different and high-quality characteristics from their parents (mother and father) (Hayward et al., 1993; Kantoğlu, 2014a). For example; yield, quality, resistance to viral and fungal diseases, resistance to pests, adaptability to environmental conditions, etc. While the most of companies selling F_1 seeds in Türkiye in the past were foreign companies, the number of domestic companies has been increasing day by day with the increase in the number of qualified personnel working in plant breeding. With the presence of local genotypes and improving new varieties seeds in many vegetable species in Türkiye, the demand for foreign companies is gradually decreasing, and thanks to local companies in the country, the production of many types of hybrid seeds improved in many different varieties.

In the world and in Türkiye breeding studies began with classical breeding and tissue culture methods (Abak, 1983) and today continue with more advanced technologies. In order to eliminate the negative effects that still occur in varieties obtained with different methods (classical breeding, anther culture, biotechnological methods, etc.), researchers have tried to create new genetic variations using an alternative method; different physical and chemical mutagens (agents that change the genetic structure of an organism) (Kökpınar et al., 2021).

Physical and chemical agents that artificially initiate hereditary changes to create variation in plants increase genetic diversity in plants and help to create new mutant lines with improved characteristics (Krupa-Małkiewicz et al., 2017). While physical mutagens cause more chromosome changes and larger DNA deletions, chemical mutagens usually cause point mutations (Okagaki et al., 1991; Anonymous, 2024a).

More than 70% of the mutant varieties obtained and sold in the market to date have been obtained by using physical mutants. The most commonly used physical mutation source has been gamma rays. Mutation breeding was first performed in pepper in 1940 using X-rays (Daskalov, 1986). In later years, many researchers obtained genetic variations using different physical and chemical mutagens (Micke and Donini, 1993; Lambat et al., 2012; Sikder et al., 2013; Sikder et al., 2015; Cheng et al., 2019; Dhamayanthi and Reddy, 2000; Xu et al., 2020; Soyam, 2021; Tanaka et al., 2021; Maurya and Bahadur, 2022; Dongfu et al., 2022; Singh et al., 2022). Mutation breeding, which is a method frequently used by plant breeders, is known to be an important method in terms of its lower cost, providing a large number of genetic variations, and especially in the development of characters that show simple heredity in self-pollinated plants (Sagel et al., 2002; Gerami et al., 2017). The frequency of mutation that will occur with physical or chemical mutagen application can be 103 times higher than the mutation frequency that will occur naturally (Kantoğlu, 2014b). However, in mutation breeding, the emergence of new lines and their detection can take a long time and with great difficulty. Since mutation breeding also occurs at low frequencies, if a large number of plant materials (thousands of plants) are started, the formation of a new and desired character is possible (Anonymous, 2024b).

As a result of studies using different physical and chemical mutation methods, many species and varieties with the desired characters (dwarfism, high yield and quality, resistance to diseases and pests, etc.) have been developed by many public and private companies in the world and in Türkiye (Tantray et al., 2017; Kazaz and Kholmurotov, 2022). Mutation breeding, due to its easy applicability, non-hazardous and environmentally friendly, fast and proven breeding method, has been commercially used in more than 210 plant species in more than 70 countries and more than 3.400 mutant plant varieties. The product with the most mutant variety production in the world is rice (873 units), followed by barley (307 units), chrysanthemum (285 units), wheat (265 units) and soybean (182 units) is the fifth plants. The Asian region ranked first with 2.087 mutant varieties produced, followed by Europe with 960, North America with 211, Africa with 82, and Latin America with 53 mutant varieties. The most developed country in the world with mutant varieties was China. China, which had 817 mutant varieties, was followed by Japan with 500 varieties and India with 345 varieties (Anonymous, 2024c). The number of mutant pepper varieties produced and registered in the world was 16 (Table 2). In Türkiye, mutant varieties were registered in 19 different plant species (Anonymous, 2024d). When table 2 was examined, it was seen that there was no registered variety in our country, but it was seen in the literature that many studies had been carried out since ancient times under the leadership of Türkiye Atomic Energy (Tepe et al., 2003; Taner et al., 2004; Beşirli et al., 2007; Kantoğlu et al., 2010; Kantoğlu et al., 2014a-b, Sarıçam et al., 2017; Kantoğlu et al., 2018; Büyükdinç et al., 2019; Aziz et al., 2021).

Table 2. Mutant pepper varieties produced in the world.

Variety Name	Latin Name	Common Name	Country Name	Registration
Albena	*Capsicum annuum* L.	Green pepper	Bulgaria	1976
F_1 Orange Beauty	*Capsicum annuum* L.	Vegetable Pepper	Russian Federation	2011
Friar KS80	*Capsicum annuum* L.	Green pepper	Italy	1985
Gornooriahovska door	*Capsicum annuum* L.	Pepper	Bulgaria	1997
Horgoska slatka-X-3	*Capsicum annuum* L.	Pepper	Serbia	1974
Krichimsky ran	*Capsicum annuum* L.	Green pepper	Bulgaria	1972
Ljulin	*Capsicum annuum* L.	Green pepper	Bulgaria	1982
Longjiao 9	*Capsicum annuum* L.	Pepper	China	2005
MDU.1	*Capsicum annuum* L.	Chilli	India	1976
Nush-51	*Capsicum annuum* L.	Sweet pepper	Russian Federation	1991
Orangeva Door to door	*Capsicum annuum* L.	Sweet pepper	Bulgaria	1991
Rice	*Capsicum annuum* L.	Sweet pepper	Bulgaria	1991
Yujiao 1	*Capsicum annuum* L.	Pepper	China	2002
Yujiao 2	*Capsicum annuum* L.	Pepper	China	2006
Yujiao 3	*Capsicum annuum* L.	Pepper	China	2007
Yujiao 4	*Capsicum annuum* L.	Pepper	China	2007

Source: Anonymous, 2024c.

The plant species produced in Türkiye and registered by the IAEA are shown in Table 3. It has been seen that most of the studies were on fruit species in Türkiye.

Table 3. Plant species recorded by the IAEA in Türkiye.

Variety Name	Latin Name	Common Name	Country Name	Registration
Bademler Beyazı	*Chrysanthemum x morifolium Ramat*	Bademler Beyazı	Türkiye	2022
Ege Meltemi	*Chrysanthemum x morifolium Ramat*	Chrysanthemum	Türkiye	2022
Kaan	*Chrysanthemum x morifolium Ramat*	Chrysanthemum	Türkiye	2022
Ozan	*Chrysanthemum x morifolium Ramat*	Chrysanthemum	Türkiye	2022
Nahita	*Solanum tuberosum* L.	Potato	Türkiye	2016
Önder	*Hordeum vulgare* L.	Barley	Türkiye	2016
Aldamla	*Prunus avium* L.	Cherry	Türkiye	2014
Burak	*Prunus avium* L.	Sweet cherry	Türkiye	2014
Birkan	*Sesamum indicum* L.	Sesame	Türkiye	2011
ALATA	*Citrus limon* L. Burm.	Lemon	Türkiye	2010
Gülşen	*Citrus limon* L. Burm.	Lemon	Türkiye	2010
Uzun	*Citrus limon* L. Burm.	Lemon	Türkiye	2010
Eylül	*Citrus limon* L. Burm.	Lemon	Türkiye	2009
TAEK-SAGEL	*Cicer arietinum* L.	Chickpea	Türkiye	2006
TAEK-PESKIRCIOGLU	*Nicotiana tabacum* L.	Tobacco	Türkiye	1999
TAEK-TUTLUER	*Nicotiana tabacum* L.	Tobacco	Türkiye	1999
Akdeniz M-Q-54	*Hordeum vulgare* L.	Barley	Türkiye	1998
TAEK A3	*Glycine max* L.	Soybean	Türkiye	1994
TAEK C10	*Glycine max* L.	Soybean	Türkiye	1994

Anonymous, 2024c

It is seen in the examination of academic studies that many studies have been conducted on the use of mutation breeding in peppers in the world. Although the number of pepper varieties registered and recorded by the IAEA seems to be low, it is known that there are still many studies in the breeding process around the world. In order to determine the targeted characteristics in the studies, it is very important to create the variation to obtain with the correct dose of mutagen (physical or chemical) applications. It should not be forgotten that the LD^{50} dose and dose limits can be vary for each genotype to genotype (Spencer-Lopes et al., 2018; Çelik et al., 2021). In order to develop an effective variation, determining the LD^{50} mutagen dose specific to each variety or genotype before starting the mutation breeding project increases the success of the study (Kantoğlu, 2022). Some of the academic studies on mutation breeding in pepper are as follows.

Dongfu et al. (2022), in pepper (*Capsicum annuum*) used EMS chemical mutagens to increase genetic variations. At the end of the study, it was reported that different structural variations were obtained at different doses. Soyam (2021) used different doses of EMS (0.2, 0.3 and 0.4%) to increase chlorophyll in pepper and ascorbic acid content, it was stated that as the EMS doses increased, the ascorbic acid content decreased and the amount of chlorophyll-a increased. Tanaka et al. (2021) applied EMS to obtain seedlessness in pepper and stated that they obtained seedless lines at the end of the study. Cheng et al. (2019) applied ethyl methane sulfonate to determine the physico-biochemical characterization of leaf color in pepper. At the end of the study, it was stated that leaf colors deteriorated according to the doses. Dhamayanthi and Reddy (2000) applied gamma rays (15, 25, 35 kR) and EMS (They investigated the effects of 0.8% and 1%) applications on the plant. EMS has been reported to be more effective in inducing meiotic irregularities than gamma rays. Lambat et al. (2012) reported that gamma rays (10 KR, 20 KR and 30 KR) and ethyl methane sulfonate (0.1%, 0.2% and in their studies investigating the cytological and morphological effects of 0.3%) on pepper, they reported that it induced various nuclear and chromosomal abnormalities. Sikder et al. (2015) reported that they used gamma rays and EMS mutagen on tomatoes, and that as a result the increase in both mutagens, seed germination, seedling height and number of pollen decreased. As a result of there was a decrease in productivity too. Sood et al. (2016) applied gamma ray (0.5 kR, 1.0 kR, 3.0 kR, 5.0 kR, 8.0 kR, 11 kR, 13 kR, 16 kR, 19 kR and 22 kR) and EMS (0.1%, 0.25%, 0.50%, 0.75%, 1.0%, 1.25%, 1.5%, 1.75%, 2.0% and 3.0%) doses, there were decreases in germination, root length, shoot length and seed emergence rate. It has been stated in the studies that UV rays are also used in mutation breeding (Anonymous, 2024e) Rodríguez-Calzada et al. (2019) UV -B radiation in hot pepper (*Capsicum annuum* L.) on morphology, phenolic compound production, gene expression and related drought stress responses, they reported that UV-B reduced the length of stalk, stalk dry weight and number of flower primordia in pepper. Rajashekara et al. (2021) Ultraviolet- In their study examining the effect of C radiation on the genetics and biochemical composition of capsicum plants, they reported that they found minimum amounts of protein, carbohydrates and free fatty acids in plants with very high mutation rates. Lee et al. (2014) used UV-ABC rays to study the mutated lettuce (*Lactuca sativa* L.) growth and phenolic compounds, they reported that repeated types of UV light increased the phenolics in the plant; however, it seriously inhibited the growth of lettuce.

By following these positive developments in mutation breeding, this study aimed to determine the germination rates and the "Effective Dose of Mutation (EMD_{50})" by using two different mutagens (gamma ray and Ethyl Metan Sulfonate chemical) applying to three different pepper varieties (Çermik genotype, Üçburun and Kandil).

MATERIALS AND METHODS

Materials

The plant material used in the study were Çermik pepper, Üçburun and Kandil bell pepper. The seeds of these material used as propagation material. Çermik is a local genotype and grown in Çermik district and Diyararbakır. This genotype also has geographical indication registration certificate and the seeds were taken from Diyarbakır Agricultural Research Institute which selected in recent breeding programme. Üçburun and Kandil bell pepper varieties were also purchased in sealed packages from the authorized dealer of Antalya-based company.

Ethyl Methane Sulfonate as a chemical mutation material supplied from the company of Sigma-Aldrich. Co^{60} source (power 2.07 kGy/h) from physical mutations was irradiated at TENMAK institution.

Methods

This study was carried out in the laboratory and greenhouse of the Department of Horticulture, Faculty of Agriculture in Dicle University. The seeds used in the experiment were sown in laboratory and in greenhouse conditions.

Chemical teratments

The seeds of the plant materials used in chemical trial were kept in containers filled with tap water for 12 hours before EMS application. Different doses of EMS (Lambat et al., 2012; Devi and Selvakumar, 2013; Pharmawati et al., 2018; Soyam, 2021) were prepared (0%, 0.1%, 0.2%, 0.3%, 0.4%, 0.5%, 0.75% and 1%) and 500 seeds were put into each glass beaker; the seeds of the control group (0 dose) were also placed in glass beaker filled with tap water again. For a total of 6 hours and 9 hours, petries with closed lids were shaken for 5 minutes/ hour for getting homogeneous treatment. At the end of 6 and 9 hours (Tantray et al., 2017; Akalp and Pirinç, 2024), these seeds were washed thoroughly under tap water three times at 20 minute intervals and then sown in petries.

Physical teratment

The seeds, which were exposed to gamma radiation by the authorities at the TENMAK office affiliated to the Turkish Atomic Energy Agency, were sown when they arrived at Dicle University in the evening of the same day. For gamma irradiation, 8 different doses of 0, 50, 100, 200, 300, 400, 500 and 600 Gy were applied, recent studies used Co^{60} source (Hayward et al., 1993; Tepe et al., 2003). The seeds exposed to mutagens and in control group were sown in 45’ viols with a 2:1 (peat:perlite) mixture. After all sowing were completed, greenhouse temperatures were kept at 25 ºC in day and 22 ºC at night.

Establishment of experiment

The experiment was set up with 5 replications for each mutation application and 45 seedlings were used in each replication. For germination tests, the experiment was set up with 4 replications in petri dishes in plant growth chamber and each replication included 100 seeds. The germinated seeds were counted on the 7th, 14th, 21st, 28th and 35th days after sowing. Seeds having 2 mm radicle were accepted as germinated. Germination rates were determined with the number of seeds counted on the last day (35th day). 45 days after sowing, seedlings were taken out of the viols and washed under tap water to determine the effective dose of mutation. In the experiments some measurements and observations were taken such as germination percentages of EMS and gamma rays, height of seedlings, for each variety and applications.

The obtained data were analyzed using variance analysis in the IBM SPSS v25 (2024) statistical package program factorial experimental design and linear regression analysis were done with Excel Microsoft program.

RESULTS AND DISCUSSION

After the application of EMS doses on the seeds of three pepper varieties for different periods, germinated seeds counted and the statistical results were given in Table 4. According to the Table 4, germination rates were decreased in all pepper varieties while compared with control group. The effect of EMS applied at different doses for 6 hours and 9 hours on the germination rates of seeds of Kandil variety were statistically significant. While the highest germination rate of 86%; was obtained in control group but the lowest germination rate was O with the dose of 1% for 9 hours application. Similar result were also for Üçburun variety; while the highest germination rate was obtained in control group with 77%, but the lowest germination rate of (O) obtained in 1% doses.
Çermik variety even though being a local genotype have highest germination rate of 92% in the seeds of control group and the lowest germination rate was 29% in 9 hours applications of 1%.

Table 4. Germination percentages of seeds 35 days after EMS application

	KANDİL		ÜÇBURUN		CERMIK	
	6 HOURS	9 HOURS	6 HOURS	9 HOURS	6 HOURS	9 HOURS
Applications	Germination rate (%)	Germination rate (%)	Germination rate (%)	Germination rate (%)	Germination rate (%)	Germination rate (%)
Control	80.50±3.79 a	85.50±2.31 a	74.00±1.37 a	77.00±2.91 a	91.50±1.65 a	90.50±2.90 a
0.1%	79.00±3.53 a	59.00±1.19 a	71.50±2.07 ab	57.50±0.40 b	88.00±0.87 ab	88.50±4.52 a
0.2%	77.00±2.41 a	56.50±3.90 ab	70.00±1.74 abc	54.50±1.24 b	91.00±0.58 a	88.00±0.43 a
0.3%	63.00±5.93 ab	43.50±3.77 bc	62.50±2.15 bc	52.00±0.77 b	87.00±1.04 ab	87.50±5.00 a
0.4%	44.50±2.58 bc	39.00±2.69 cd	58.50±1.46 cd	36.00±0.59 c	81.00±0.58 bc	86.50±2.20 a
0.5%	36.50±4.14 cd	28.50±4.01 d	56.00±2.52 cd	31.50±3.89 c	78.50±1.02 c	86.00±2.33 a
0.75%	33.50±4.52 cd	0.50±0.80 e	56.00±1.02 d	0.00±0.83 d	69.00±3.27 d	35.50±1.72 b
1.0%	22.00±4.12 d	0.00±1.24 e	41.50±7.16 e	0.00±0.83 d	55.00±2.12 e	29.00±2.23 b
p value	<,0001	<,0001	<,0001	<,0001	<,0001	<,0001
CV	15.75	16.23	12.38	10.31	4.37	8.68
LSD	33.29	35.3	32.13	37.86	17.72	36.06

The effect of different doses of gamma rays on germination rate for the seeds of three different pepper varieties were statistically significant as shown in Table 5. As the dose of gamma rays increased, the germination rate were decreased for all three groups. While the highest germination rate of 93% was obtained in control group of Kandil variety, the lowest germination rate of 9.50% was obtained at 600 Gy dose. A similar result was observed in Üçburun variety; while the highest rate (93.50%) was obtained in the control group, but 600 Gy dose have the lowest germination rate of 16%. In Çermik genotype the difficulties in germination can be explained, by incerasing of dose cause to decrease the germination rate. As the highest germination rate was obtained in the seeds of control group (94%), the lowest germination rate (47%) obtained at the highest dose of 600 Gy.

As a result of the study it can be said that decreases in germination percentages as a result of increasing EMS application duration and EMS doses. A similar situation also occurred after the use of gamma rays, which are physical mutagens. As the doses of gamma rays increase, the germination rate decreases. Pharmawati et al. (2018) reported; using 0.5%, 0.75% and 1.0% EMS doses on pepper the germination and emergence of seeds decreased with increasing dose while comparing to control group.

Similar results were reported by Sood et al. (2016) in determining the lethal dose with gamma rays and EMS application on bell pepper. In the study, it was reported that high doses of gamma rays (19kR and 22kR) and EMS (2.0 % and 3.0%) caused a decrease in percentages of seed germination and less growth of seedlings. Sanjai Gandhi et al. (2014) reported that germination percentages decreased with increasing doses using ethyl methane sulfonate (10, 20, 30, 40 and 50 mM) and diethyl sulfate (5, 10, 15, 20 and 25 mM) mutagens. Arisha et al. (2014) reported that germination percentages decreased with increasing doses of EMS applied to pepper seeds (0, 0.25, 0.50, 0.75, 1.00, 1.25, 1.50, 1.75, 2.00, 2.25, 2.50% v/v). Alcantara et al. (1996) tried to determine seed mutagenesis in pepper using 0.5%, 1.0% and 1.5% EMS doses and seeds exposed to 5°C, 10°C, 15°C and 20°C for 3, 6 and 9 hours.

They stated that seeds treated with 1.5% EMS for 9 hours at 20°C in the M1 generation had the lowest germination percentage among 36 treatments. Jabeen and Mirza (2002) reported that germination decreased with increasing EMS (0.01, O.1, O.5 % VN) concentrations in 3 and 6 hour applications in order to increase genetic variations in pepper. It was reported that the lowest germination was obtained with 0.5% EMS application for 6 hours. Saba and Mirza (2002) tried to determine genetic variations in pepper by using 0.1%, 0.5% and 1% ethyl methane sulfonate (EMS) doses for 3 and 6 hours. It was reported that germination rates decreased with increasing doses.

Table 5. Germination percentages of seeds 35 days after gamma ray application.

	KANDİL	ÜÇBURUN	CERMIK
Applications	Germination rate (%)	Germination rate (%)	Germination rate (%)
Control	93.00±1.58 a	93.50±0.95 a	93.50±1.18 a
50	81.50±0.54 b	79.75±1.58 b	87.00±0.72 b
100	80.00±0.60 b	74.25±0.69 c	86.50±0.24 b
200	75.00±0.72 bc	72.75±0.58 cd	84.50±0.24 b
300	71.00±1.97 c	68.50±0.98 d	79.25±0.74 c
400	49.50±1.59 d	51.00±1.73 e	75.00±0.87 c
500	16.00±1.95 e	19.50±0.20 f	55.75±0.48 d
600	9.50±1.47 e	16.00±0.70 f	46.75±1.59 e
CV	5.10	3.75	2.45
p value	<,0001	<,0001	<,0001
LSD	42.98	38.72	22.52

The effect of physical mutation (gamma) doses applied to different types of pepper seeds on plant height during the seedling period is given in Table 6. According to the table, the effect of gamma ray application on seedlings height was statistically significant for all three types of pepper seeds.

Table 6. Effect of different Gamma doses on seedling height.

	KANDİL	ÜÇBURUN	CERMIK
Applications		Seedling height (cm)	
Control	10.71±0.22 a	9.19±0.14 b	12.60±0.46 a
50	9.10±0.22 b	8.09±0.13 c	10.15±0.32 b
100	6.05±0.13 c	7.15±0.13 c	8.18±0.22 c
200	4.97±0.07 d	6.94±0.13d	6.68±0.21 d
300	4.09±0.07 e	5.93±0.13e	6.76±0.30 cd
400	4.00±0.07 e	4.85±0.22 ef	4.76±0.24 e
500	3.91±0.07 e	4.08±0.22 f	4.00±0.32e
600	3.79±0.07 e	3.68±0.22 a	3.39±0.29 e
p value	<,0001	<,0001	<,0001
CV	9.420976	11,2007	14.62497
LSD	4.71	3.59	5.4

In terms of plant height, which is the most important parameter in determining the effective mutation dose in mutation breeding, after gamma irradiation, the highest height was found in control group in Kandil, Üçburun and Çermik pepper seedlings respectively; 10,71 cm, 9,19 cm and 12,60 cm. As the doses increased, decreases were seen in these values compared to the seedlings of control group (Kandil: 3,79 cm; Üçburun: 3,68 cm; Çermik: 3,39 cm). According to the results of the statistical analysis; EMD_{50} dose was calculated as 315,49 Gy in Kandil variety as a result of gamma application. EMD_{50} of the Kandil variety is given in figure 1.

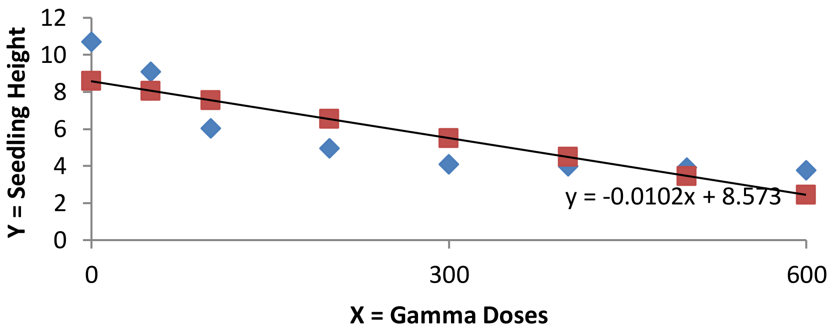

Figure 1. Change in seedling heights of Kandil variety as a result of the application of gamma doses

The figure showing the EMD_{50} as a result of applying different doses of Gamma to the ÜB variety is given below (Figure 2). The EMD_{50} dose in the ÜB variety was determined as 454.95 Gy.

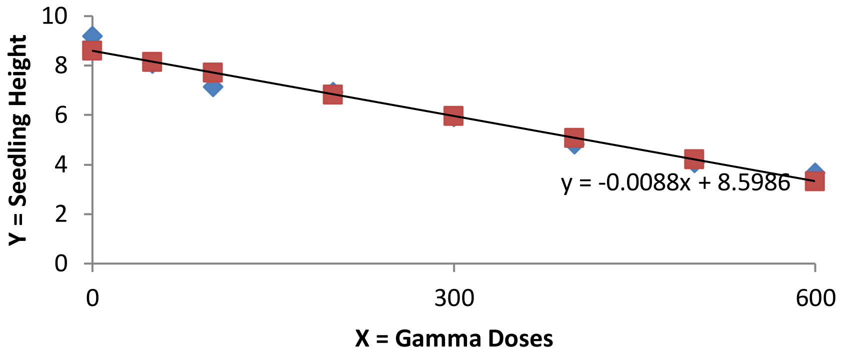

Figure 2. Change in seedling heights of Üçburun variety as a result of the application of gamma doses

In Çermik genotype, the EMD_{50} dose was determined by detecting abnormal shortening in the seedlings as a result of the application of Gamma rays. In Çermik genotype, EMD_{50} was determined 329.26 Gy due to Gamma irradiation (Figure 3).

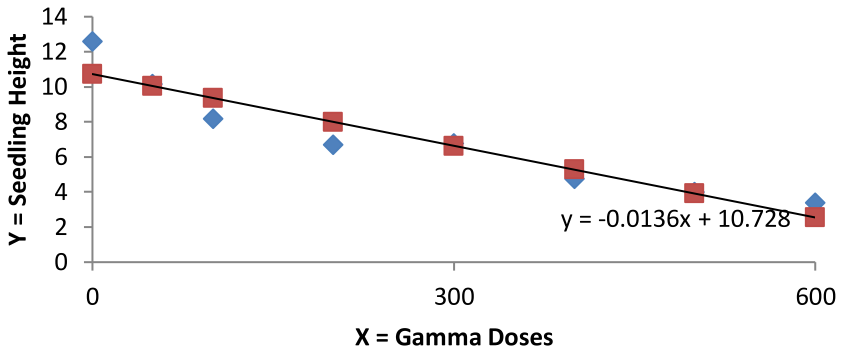

Figure 3. Change in seedling heights of Çermik variety as a result of the application of gamma doses

As mentioned in the studies, there are different mutation doses among vegetable species and varieties. Kantoğlu (2022) reported that there were decreases in plant height with increasing gamma doses in his study to determine the EMD_{50} dose in watermelon. In addition, Puripunyavanich, (2003), Velkov et al., (2016); Ernest et al., (2020), Çelik et al., (2021), Kantoğlu and Kunter, (2021), Zafar et al., (2022) reported decreases in seedling heights in parallel with increasing radiation doses in their studies. Tepe et al., (2003) reported that shoot development decreased at doses of 200 Gy and that there were especially significant decreases at the dose of 400 Gy to find the effective mutation dose in pepper. Micke and Donini (1993) also stated that doses of 140-220 Gy reduced shoot development. Sood et al., (2016) reported that seedlings'length decreased in bell pepper at high doses of gamma rays (19 kR and 22 kR). Kökpınar et al., (2024) reported that for lettuce, plant height decreased as the dose of gamma rays increased and the decreases were especially evident at the dose of 200 Gy.

The parameters shows the effects of different EMS doses on three different pepper seedlings were given in Table 7. When table is examined, it was determined by examining the results the use of EMS doses in pepper mutation breeding is important.

Table 7. Effect of Different EMS Doses Applied at Different Times on Seedling height

	Kandil	ÜB	Cermik	Kandil	ÜB	Cermik
	6 Hours			9 Hours		
Applications	Seedling height (cm)			Seedling height (cm)		
Control	13.53±0.18 a	10,29±0,25 a	14,65±0,57 a	13.61±0.11 a	11.46±0.27 a	13.75±0.43 a
0.1%	10.49±0.19 b	9,63±0,16 ab	14,11±0,58 a	9.69±0.13 b	9.58±0.13 b	13.22±0.36 a
0.2%	10.09±0.13 b	9,01±0,13 bc	12,58±0,54 ab	8.65±0.15 c	8.83±0.08 b	11.70±0.34 b
0.3%	9.15±0.12 c	8,44±0,19 cd	12,59±0,53 ab	7.75±0.13d	7.79±0.20 c	11.68±0.40 b
0.4%	7.65±0.14d	8,14±0,17 cd	11,58±0,45 bc	7.34±0.16 de	7.69±0.23 c	9.90±0.23 c
0.5%	7.23±0.18 d	7,55±0,32 de	11,65±0,41 bc	6.86±0.19 e	6.95±0.18 cd	9.49±0.20 cd
0.75%	5.46±0.09 e	6,91±0,16 e	9,83±0,24 cd	4.91±0.10 f	6.62±0.15d	8.29±0.28 d
1.0%	4.34±0.09 f	4,90±0,22 f	7,84±0,20 d	3.88±0.10g	4.63±0.27 e	6.45±0.18 e
p value	<,0001	<,0001	<,0001	<,0001	<,0001	<,0001
CV	8.21	8,62	13,20	8.34	8.44	10.08
LSD	4.71	3,59	4,35	5.53	0.84	4.39

The differences in plant heights was statistically significant as a result of application of EMS doses and different durations. In Kandil, Üçburun and Çermik pepper seedlings, the highest height was determined in control group seedlings (6-hour application: Kandil: 13,53 cm, Üçburun: 10,29 cm and Çermik: 14,65 cm; and 9-hour application: Kandil: 13,61 cm, Üçburun: 11,46 cm and Çermik: 13,75 cm); as the doses increased, decreases were obtained in these values compared to the seedlings in the control group (the lowest heights were at 1.0% doses). It has also determined in recent studies; there were shortenings in plant heights measured after the application of EMS in pepper (Kantoğlu et al., 2014; Pharmawati et al., 2018; Akalp and Pirinç, 2024). Pharmawati et al. (2018) reported that as a result of the application of ethyl methane sulfonate (0,5%, 0,75% and 1,0% EMS) in pepper, plants were stunted in the M2 generation. Sood et al., (2016) tried to determine the LM_{50} dose using gamma rays and EMS in bell pepper. They reported that plant heights decreased with the application of 2,0% and 3,0% EMS. Jabeen and Mirza (2002) reported that the 0.5% dose reduced height of seedlings by using different doses of EMS (0,01, 0,1, 0,5% VN) in pepper.

The effect of EMS on seedling height in three different pepper types was tried to determine as EMD_{50} value. According to the measurements on the seedlings after 6 hours of application in Kandil variety, the EMD_{50} value was determined as 0,67%. The figure below shows the effective mutation dose for Kandil variety (Figure 4).

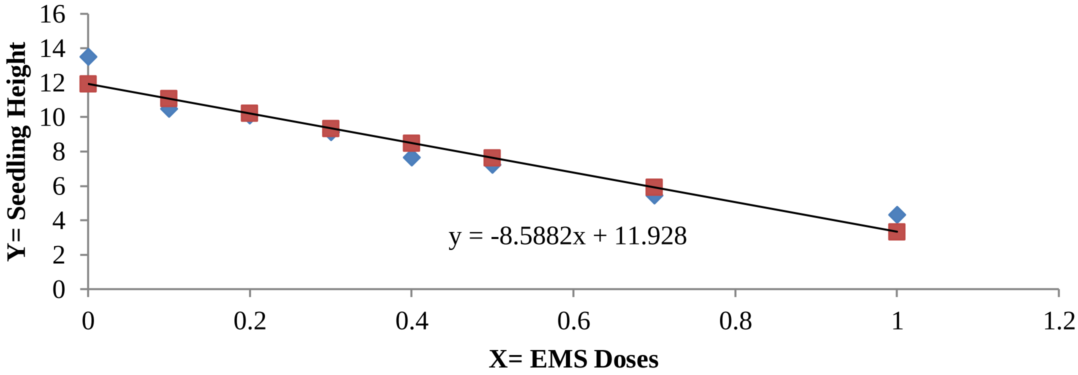

Figure 4: Determination of EMD_{50} in Kandil pepper variety after 6 hours of EMS application

The EMD_{50} determined as a result of applying different EMS doses for 6 hours to Üçburun variety is given in figure 5. According to the data obtained by measuring the height of ÜB seedlings, EMD_{50} was determined as 0.97%.

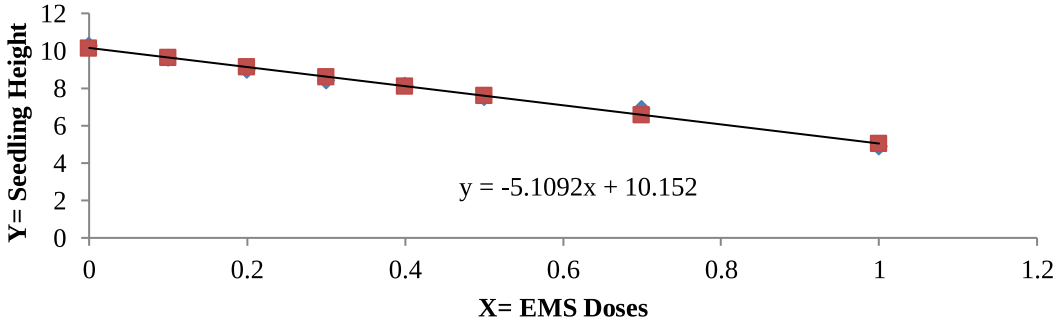

Figure 5. Determination of EMD_{50} after 6-hour EMS application in Üçburun pepper variety

The EMD_{50} determined as a result of applying different EMS doses for 6 hours in Çermik pepper genotype was given in figure 6. According to the data obtained by measuring the height of Çermik seedlings, EMD_{50} was determined as 1,08%.

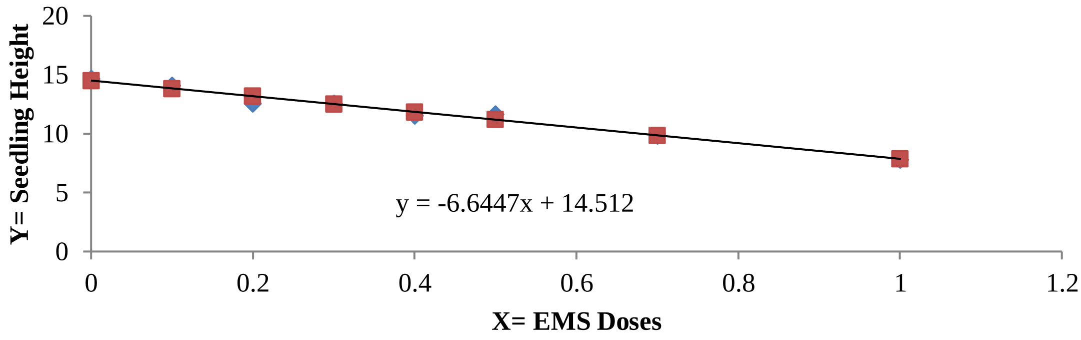

Figure 6: Determination of EMD_{50} in Cermik pepper genotype after 6-hour EMS application.

In Kandil pepper variety, the EMD_{50} value was determined as 0,52% according to the measurements of the seedlings after 9 hours of application. The figure below shows the effective mutation dose in Kandil variety (Figure 7).

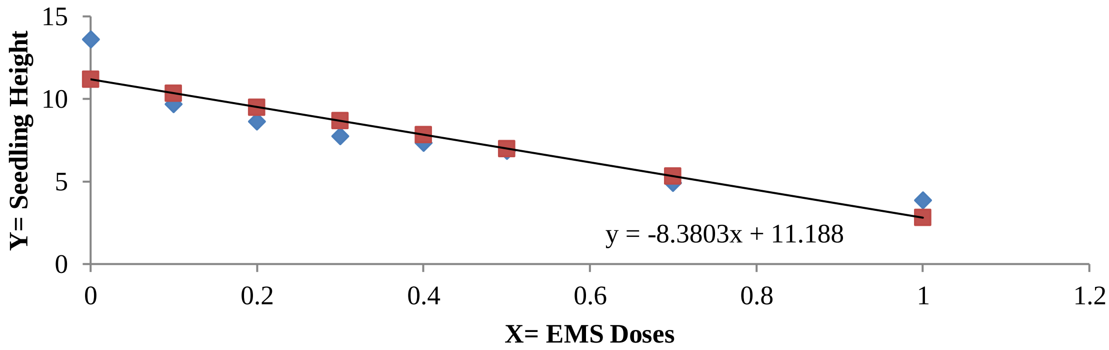

Figure 7. Determination of EMD_{50} after 9-hour EMS application in Kandil pepper variety.

The EMD_{50} determined as a result of applying different EMS doses for 9 hours to Üçburun pepper variety was given in figure 8. According to the data obtained by measuring the height of Üçburun seedlings, EMD_{50} was determined as 0,77%.

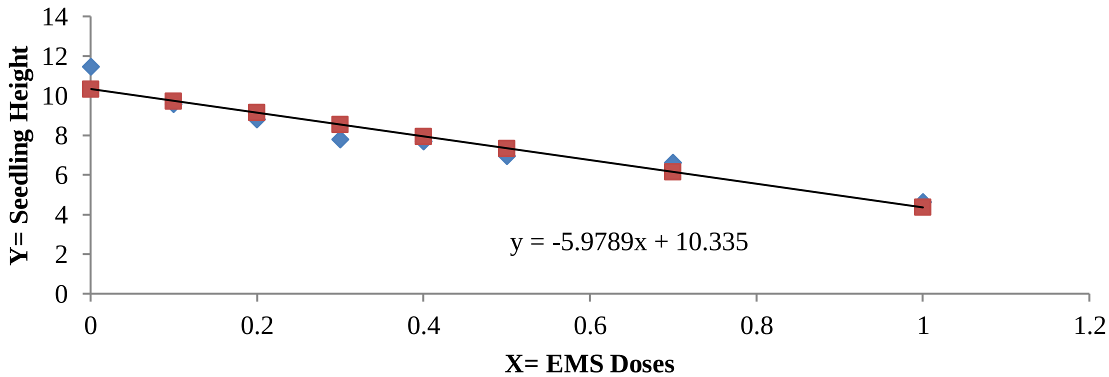

Figure 8: Determination of EMD_{50} after 6-hour EMS application in Üçburun pepper variety.

The EMD_{50} determined as a result of applying different EMS doses for 9 hours in Çermik pepper genotype was given in figure 9. According to the data obtained by measuring the height of Çermik seedlings, EMD_{50} was determined as 0,89%.

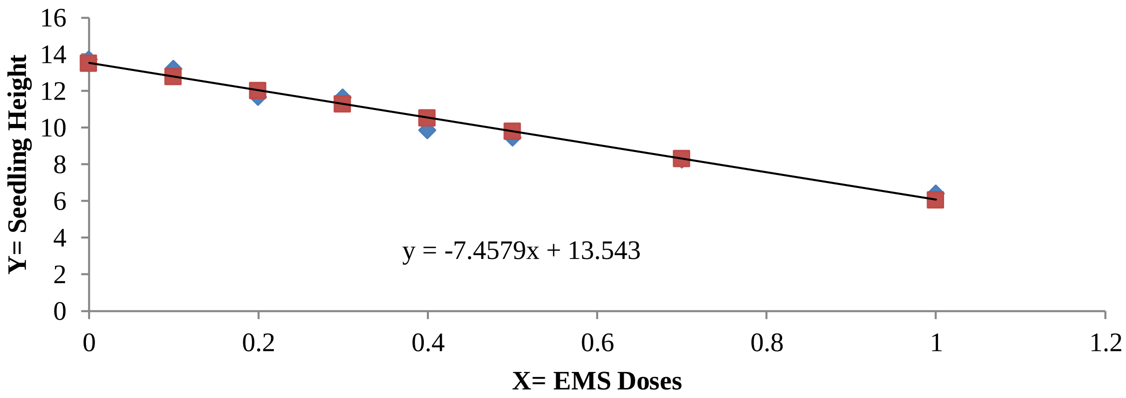

Figure 9: Determination of EMD_{50} in Cermik pepper genotype after 9 hours of EMS application.

CONCLUSION

As the doses of physical and chemical mutagens, which are among the mutation breeding methods, increase, they create physiological and morphological changes in the plant. Mutation breeding methods, which plant breeders frequently use to create variations in shorter time with lower cost while compared to other method. And also provide the opportunity to create new variety candidates with desired characters in many plant species. At the end of the study, EMD_{50} in three different pepper genotypes as a result of CO^{60} irradiation, one of the gamma ray types, was Kandil: 315.49; for Üçburun: 454.95 and Çermik: 329.26 Gy. The results obtained from Kandil bell and Üçburun pepper varieties, which respond to the lower and upper limits of gamma ray dose, gave similar results to the doses applied to other commercial varieties in academic studies. In the study, the application of different EMS doses gave similar results for three different pepper seeds. At the end of the study, it was observed that with increasing EMS doses, there were decreases in the "plant height" parameter, which is used as a reference parameter in determining the effective mutation dose. The results of this study were parallel to other academic studies were obtained, with different doses of EMS applied to three different pepper seeds, the EMD_{50} value in pepper was between 0,67% and 1,08% in 6 hours of application. And It was found between 0,52% and 0,89% for 9 hours of application. It was determined that the mutation frequency increased with the increase in application times; but it was determined that the EMD_{50} value also decreased.

In this study, in which different physical and chemical mutagens were used, applications were made to three different pepper varities, the effect of mutagen doses on seed germination and pepper seedlings were investigated, and results of this research will be reference to future studies on mutation breeding. The later level of this study will serve new breeding lines for new varieties.

Compliance with Ethical Standards

Peer-review

Externally peer-reviewed.

Declaration of Interests

There is no conflict of interest in the article

Author contribution

Each author has the equal contributiıon (for each 50%)

Ethics committee approval

There is no need for ethichs committee approval.

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