TURKISH ENERGY, NUCLEAR AND MINERAL RESEARCH AGENCY
TURKISH JOURNAL OF NUCLEAR SCIENCES
E-ISSN: 2791-7185
https://dergipark.org.tr/tr/pub/tjns

Mutation dose determination of cherry tomato (*Solanum lycopersicum L.* var. *cerasiforme*) under *in vitro* and *ex vitro* conditions

Irmak Çakın[1,*], **Kadriye Yaprak Kantoğlu**[1], **Erhan Aksu**[1]

[1] *Nuclear Energy Research Institute of the Turkish Energy, Nuclear and Mineral Research Agency, ANKARA, 06980, Türkiye*

ARTICLE INFO

Article History:
Received May 29, 2025
Available online December 31, 2025

Research Article

Keywords:
Effective mutation dose (EMD$_{50}$)
Solanum lycopersicum L. var. *cerasiforme*
in vitro
ex vitro
Cherry tomato

ABSTRACT

In this study, biological responses resulting from gamma irradiation of cherry tomato (*Solanum lycopersicum* L. var. *cerasiforme*) seeds under different germination conditions (*in vitro* and *ex vitro*) were evaluated, and the effective mutation dose (EMD$_{50}$) was determined. In the *in vitro* experiment, seeds were irradiated with gamma rays at doses ranging from 0 to 80 Gy and germinated on Murashige and Skoog (MS) medium without any plant growth regulators. In the *ex vitro* conditions, seeds were exposed to doses between 0 and 600 Gy and sown in pots filled with a soil mixture. Seed germination was monitored during the first 10 days post-irradiation, and parameters such as shoot length, fresh weight, and dry weight of seedlings were measured at the end of a 30-day incubation period. Regression analyses revealed an EMD$_{50}$ value of 39,08 Gy under *in vitro* conditions and 608,6 Gy under *ex vitro* conditions. The findings demonstrate that the effective irradiation dose varies depending on the irradiation conditions (*in vitro* vs. *ex vitro*). These results emphasize the importance of considering the culture conditions when selecting the appropriate irradiation dose in mutation breeding programs in cherry tomato.

1. Introduction

Tomato, whose native region is the Andes Mountains, reached Europe in the 16th century and has become an economically important crop widely cultivated worldwide today [1]. Ranking 11th in global production, tomato is a significant vegetable species characterized by low sugar content [2]. It belongs to the Solanaceae family, which also includes economically important plants such as potato, eggplant, and pepper [3, 4]. Based on FAO data from 2023, global tomato production reached approximately 189 million tons [5]. Additionally, Türkiye, as a significant producer, recorded a harvest of around 13,3 million tons of tomatoes in the same year [6].

Among the approximately 3000 species in the Solanaceae family, tomato (*Solanum lycopersicum* L.) stands out due to its significant contribution to human nutrition. The tomato species contain varying amounts of essential nutrients such as sugars, fibres, proteins, amino acids, and cholesterol-free lipids, and it offers important health benefits not only because of its nutritional value but also due to its bioactive compounds including carotenoids, vitamin C, and provitamin A [7-10]. Some of these compounds are known to have the potential to prevent certain types of cancer. Lycopene, a natural antioxidant found in tomatoes, has attracted attention as a cancer-preventive agent. It has been found that lycopene is inversely correlated with the incidence of certain cancers, particularly prostate cancer [11].

Additionally, tomatoes are particularly rich in carotenoids and phenolic compounds, which contribute to their antioxidant capacity. Tomatoes are also a rich source of potassium and various carboxylic acids. Improving tomato flavour poses a significant challenge due to the complex composition of numerous compounds related to the fruit's taste and aroma. The fruit flavour is closely associated with the concentrations of sugars such as glucose, fructose, and sucrose, as well as organic acids, primarily citric and malic acids. On the other hand, the aromatic profile is shaped by the diversity of volatile organic compounds (VOCs) and their interaction with taste and texture; all these factors together form the overall flavour perception as sensed through both taste and retronazal olfaction [12]. Each of these traits is controlled by multigenic systems, resulting in a physiologically complex structure. This represents a critical point for breeding efforts aimed at developing new varieties that meet the ever-changing demands of consumers. Considering only aroma components, although more than 400 volatile compounds have been identified in tomatoes, fewer than approximately 10% are considered organoleptically (sensory) significant [13].

---

*Corresponding author: irmak.cakin@tenmak.gov.tr

Mutations commonly utilized in the agricultural industry, such as self-pruning, ovate fruit shape, and jointless pedicel structure, enable the development of plant types suitable for mechanical harvesting, characterized by compact architecture, determinate growth, and elongated fruits. Additionally, mutations like fasciation-characterized by abnormal widening and flattening of tissues in stems, flowers, or fruits-and genetic changes that increase the number of locules (seed chambers) significantly influence fruit size and play an important role in breeding programs aimed at fresh consumption. Beyond its economic importance, tomato (*Solanum lycopersicum* L.) is considered a model species among fleshy-fruited plants due to its short life cycle, self-pollination capability, and resilience to biotic and abiotic stresses [14]. The tomato species holds a key position within the Solanaceae family, which is globally cultivated and economically significant. In addition to being an important source of essential nutrients, it serves as a model for the transfer of important genes to other dicotyledonous crops. Whether consumed fresh or processed, tomato is widely used in research owing to its short lifespan, small genome, and simple diploid genetics. However, it faces challenges such as sensitivity to various stress factors. To overcome these difficulties, breeders aim to enhance genetic diversity by developing varieties with desired traits through genetic resources, employing methods such as hybridization, transgenic technologies, and CRISPR/Cas9 gene editing [7, 15].

Mutation breeding, one of the classical breeding methods, encompasses the process of inducing and developing mutant lines to improve plant traits. While traditional breeding methods generate new genetic combinations, mutation breeding offers an alternative approach that introduces new and desirable traits without completely disrupting the existing genetic background [16]. Gamma rays, a widely used mutagen, are highly effective in creating genetic variation. The applied mutagenesis stands out as a strategic method to enhance specific traits while preserving the existing genetic framework [15]. In the context of tomato breeding, gamma ray-induced mutants, including those related to drought tolerance and growth, have been successfully utilized [17]. Particularly, mutation breeding techniques based on gamma ray-induced mutagenesis (GRIM) have been effectively applied across various plant species, contributing to genetic diversity and enabling the development of novel plants with desired traits [18].

Tomato, regarded as a model plant species, holds a significant place in breeding programs involving the use of induced mutants. Databases such as TOMATA and the C.M. Rick Tomato Genetics Resource Centre specifically include gamma ray-induced mutants associated with drought tolerance and growth-related traits. The alternative application of induced mutagenesis facilitates the creation of mutant pools exhibiting broad phenotypic diversity [15]. Physical mutagens, including gamma rays and proton irradiation, as well as chemical mutagens like ethyl methanesulfonate (EMS), are commonly applied across various plant species [19-23]. In tomatoes, chemical mutagenesis serves as a powerful method to generate a wide diversity of mutants with high mutation frequency, whereas physical mutagens, particularly gamma rays, tend to produce large deletions and insertions (indels) that can more readily induce phenotypic changes [15]. Recently, EMS application, as a chemical mutagen, has been effectively utilized in conjunction with next-generation sequencing (NGS) technologies to detect mutations and enhance plant tolerance to abiotic stresses [24]. Nuclear-based techniques not only address potential human health concerns associated with genetically modified plants but also offer a faster breeding timeline for obtaining definitive results. Research conducted over the past fifty years has consistently demonstrated the efficacy of mutation breeding techniques, especially in self-pollinated plants. These methods have proven advantageous in improving traits controlled by single genes and simple inheritance patterns [25-27]. Mutation breeding has been successful in inducing changes in physiological traits such as earliness and continuous, efficient flowering; altering photoperiodic responses; and enhancing tolerance to abiotic stress factors as well as increased resistance to diseases [1].

Plant tissue culture is an advanced biotechnological method that enables the multiplication of plant cells, tissues, or organs in artificial nutrient media under sterile laboratory conditions. This technique allows for the rapid and controlled production of genetically identical plants, i.e., clonal propagation, while also playing a critical role in the conservation of rare or endangered species, obtaining disease-free plant material, and the production of secondary metabolites [28]. Tissue culture is also an important tool in mutation breeding studies. In particular, the regeneration of cells treated with chemical or physical mutagens in selective media enables the development of new varieties with desired traits. This approach contributes to increasing genetic diversity in a shorter time and under more controlled conditions compared to conventional breeding methods. Widely applied in sectors such as agriculture, forestry, pharmaceuticals, and cosmetics, plant tissue culture has become a cornerstone of modern plant biotechnology [28]. There is evidence that low-dose gamma irradiation enhances germination and overall plant production. The biological effects of ionizing radiation, especially gamma rays, encompass a broad-spectrum including inhibition, stimulation, mutation, and cell death [29].

The cellular mechanisms affected by physical mutagen applications under *in vitro* and *ex vitro* conditions exhibit significant differences both in terms of the level of environmental control and the intensity of cellular stress responses [30]. In *in vitro* conditions, plant cells or tissues are exposed to mutagenic agents in a sterile and controlled environment. Plant cells cultured *in vitro* are maintained on sterile nutrient media with high humidity and controlled nutrient supply. The high humidity increases the flexibility of cell membranes and organelles, allowing physical and chemical damages caused by irradiation to be buffered before directly impacting cellular structures [31]. Moreover, the abundant water molecules present in the *in vitro* environment enhance the formation of reactive oxygen species (ROS) induced by ionizing radiation, these ROS cause damage to both DNA and other cellular components. Therefore, the high humidity and water content amplify the radiation effect at the cellular level, resulting in increased mutagenic damage. However, since stress factors are isolated in this environment, cells typically exhibit a more limited and observable response. Consequently, it is possible to select cells with high regeneration capacity, facilitating the development of controlled varieties [32].

In *ex vitro* conditions, physical mutagens (e.g., gamma radiation) are applied directly to plant tissues. As a result, ROS generation and subsequent DNA damage are lower compared to *in vitro* conditions, potentially causing an imbalance in cellular repair mechanisms. At high seed moisture levels under *ex vitro* conditions, the burden on cellular defence systems increases, which may lead to dysregulation of gene expression, apoptotic (programmed) cell death, necrosis and other severe physiological responses. When environmental humidity and water content in *ex vitro* conditions are within the desired range (5–10%), the mutagenic effect of radiation on cells decreases; therefore, higher doses of irradiation are required. However, due to tissue heterogeneity, the affected cell types and their responses vary, which reduces the homogeneity of induced mutations [32]. Nevertheless, research by Çelik et al. [1] and Kantoğlu et al. [17] demonstrates that, despite intrinsic variability, it is possible to successfully develop stable mutant lines through gamma-irradiation, resulting in the generation of valuable and genetically uniform mutants.

The aim of this study is to systematically evaluate the effects of different gamma radiation doses on the development of commercially important cherry tomato (*Solanum lycopersicum* L.) seeds under *in vitro* and *ex vitro* germination conditions and to determine the effective mutation dose (EMD_{50}) values for both environments. Within the scope of the study, the inhibitory and mutagenic effects of irradiation doses on plant growth parameters were compared to identify the optimal radiation dose to be used in mutation breeding programs. Additionally, considering the decisive role of seed germination environment and conditions on mutation efficiency, the study aimed to highlight the importance of selecting appropriate irradiation parameters for achieving success and efficiency in mutation breeding.

2. Materials and Methods

2.1 Materials

An unknown cherry tomato genotype from our gene pool, purified over four generations, belonging to a commercial variety, was used in this study. One-year-old seeds, obtained annually from the same commercial line, were utilized. The cherry tomato seeds (*Solanum lycopersicum* L. var. *cerasiforme*), supplied by the Turkish Energy, Nuclear and Mineral Research Agency (TENMAK), were used to determine the effective mutation dose (EMD_{50}) under both *in vitro* and *ex vitro* conditions.

2.2 Methods

The moisture content of the tomato seeds was determined prior to irradiation to ensure uniform physiological status. Approximately 1 g of seeds was dried at 103±2 °C for 17 hours, and moisture percentage was calculated based on the difference between fresh and dry weights.

2.2.1 In vitro experiments

A total of 90 seeds were used for the *in vitro* experiments, with three replicates per dose. The seeds were initially rinsed in 70% ethanol for 5 seconds. Subsequently, they were sterilized by shaking in a solution containing 10% sodium hypochlorite (NaOCl) with 1–2 drops of Tween 20 for 10 minutes. After sterilization, the seeds were rinsed three times with sterile distilled water. The sterilized seeds were placed onto Murashige and Skoog (1969) (MS) basal medium containing 0.7% agar and 3% sucrose, adjusted to pH 5.6, and germinated in a growth chamber under a 16/8 hour light/dark photoperiod at 24±1°C.

One-week-old *in vitro* seedlings, which had an average shoot length of approximately 3 cm, were irradiated at the Turkish Energy, Nuclear and Mineral Research Agency using a Cesium-137 (Cs^{137}) gamma source with a dose rate of 9.4 Gy/min.

The gamma irradiation doses utilised in this study were selected in accordance with the recommendations outlined in the Manual on Mutation Breeding [33]. This manual advises the application of a broad and incremental range of doses to facilitate the generation of a comprehensive dose–response curve and to accurately determine effective mutation-inducing dose levels. Accordingly, the doses chosen for experimentation were 0 Gy (control), 2 Gy, 5 Gy, 15 Gy, 20 Gy, 30 Gy, 40 Gy, 50 Gy, 60 Gy, 70 Gy, and 80 Gy. This systematic approach ensured that the study could effectively assess the impact of increasing gamma radiation levels on seed germination and plant development, thereby enabling the identification of optimal doses for mutation induction within the breeding programme.

After irradiation, shoot lengths of both *in vitro* and *ex vitro* seedlings were measured on the 30th day. For biomass analysis, fresh weights of all seedlings were recorded immediately after harvesting, and dry weights were obtained after drying the samples at 70 °C until constant weight. These measurements were performed for both *in vitro* grown plantlets to characterize dose-dependent changes in vegetative growth.

In addition to shoot length, fresh and dry weights were also recorded, as biomass parameters are widely used indicators of growth suppression and radiosensitivity in gamma irradiation studies.

2.2.2 Ex vitro experiments

In the *ex vitro* experiments, a total of 50 seeds were used, with five replicates per dose. The seeds were irradiated using the same Cs^{137} gamma source (dose rate: 9,4 Gy/min) at doses of 0 (control), 50, 100, 200, 300, 400, 500, and 600 Gy. The selection of these irradiation doses followed the recommendations of the Manual on Mutation Breeding [33], which emphasizes the use of a wide dose spectrum in *ex vitro* radiosensitivity studies to generate a comprehensive dose-response profile and to determine effective mutation-inducing levels.

The irradiated seeds were sown in pots containing a soil mixture composed of garden soil, peat, and burnt farmyard manure in a 1:1:1 ratio. The pots were maintained under controlled greenhouse conditions with 70-85% humidity, a temperature of 25 ± 1°C, and a 16/8 hour light/dark photoperiod for 30 days.

Shoot lengths of the germinated seeds were measured on the 30th day. Fresh and dry weights were also recorded for each seedling. Average shoot length was used as the primary variable for EMD_{50} estimation under *ex vitro* conditions, and biomass measurements were used to confirm the dose-response pattern.

2.2.3 Statistical analysis

Each experimental group was arranged with three or five replicates. Descriptive statistical parameters, including means, standard deviations (SD), and coefficients of variation (CoV%), were calculated for all measured traits. SD values were used to quantify variability among biological replicates, while CoV% provided an additional measure of relative dispersion across treatments. Percentage reductions in shoot length, fresh weight, and dry weight were calculated relative to the non-irradiated control using the formula [33]:

$$\text{Reduction (\%)} = \frac{(\text{Control mean} - \text{Treated mean})}{\text{Control mean}} \times 100 \quad (1)$$

These descriptive metrics were used to characterize dose-dependent trends and the magnitude of physiological alterations induced by gamma irradiation.

A simple linear regression analysis was performed in Microsoft Excel to evaluate the relationship between gamma-ray dose and growth parameters under both *in vitro* and *ex vitro* conditions. The regression models and R^2 values were used to assess the strength of the dose-response relations [33].

3. Results and Discussions

3.1 Results

Tomato seed moisture content was measured before irradiation for uniform physiological status. About 1 g of seeds was dried at 103 ± 2 °C for 17 hours, and moisture percentage was calculated from the weight difference between fresh and dried seeds. The fresh and dry weights were 3 g and 1.4 g, respectively, resulting in a moisture content of approximately 53.3%.

All 90 tomato seeds germinated prior to irradiation, corresponding to a 100% germination rate. One-week-old *in vitro* seedlings, each with an initial shoot length of approximately 3 cm, were exposed to gamma radiation doses ranging from 0 to 80 Gy. Following irradiation, the seedlings were maintained under controlled *in vitro* conditions for 30 days, after which all surviving seedlings were evaluated for shoot length, fresh and dry weight (Table 1).

Table 1. Effect of gamma irradiation on shoot growth and biomass of cherry tomato (*Solanum lycopersicum* L. var. *cerasiforme*) seedlings under *in vitro* and *ex vitro* conditions.

Çalışma	Dose (Gy)	MSLV (cm)	SD	CV (%)	FW (g)	FWR (%)	DW (g)	DWR (%)
In vitro	0	13.85	2.14	15.5	45.86	0.0	2.95	0.0
	2	11.23	2.34	20.8	40	12.8	2.84	3.7
	5	12.78	2.28	17.8	47.7	-4.0	3.2	-8.5
	15	9.62	1.6	16.6	26.1	43.1	1.8	39.0
	20	9.21	1.34	14.5	33.2	27.6	2.52	14.6
	25	6.96	1.8	25.9	20.6	55.1	1.43	51.5
	30	7.65	1.16	15.2	25.2	45.1	1.85	37.3
	40	6.12	1.23	20.1	17.5	61.8	1.1	62.7
	50	4.5	1.65	36.7	14.8	67.7	1	66.1
	60	4.23	1.01	23.9	7.3	84.1	0.63	78.6
	70	3.27	0.52	15.9	4.05	91.2	0.43	85.4
	80	3.88	1.01	26.0	6.74	85.3	0.49	83.4
Ex vitro	0	24.76	7.53	30.4	60.3	0.0	4	0
	50	28.56	7.54	26.4	61.8	-2.5	2.65	33.8
	100	30.59	7.58	24.8	36.5	39.5	1.94	51.5
	200	28.79	7.67	26.6	48.15	20.1	2	50.0
	300	26.2	6.28	24.0	56.2	6.8	3.07	23.3
	400	22.09	4.83	21.9	39.95	33.7	1.85	53.8
	500	12.08	4.5	37.3	38.35	36.4	2.03	49.3
	600	9.75	3.17	32.5	17.6	70.8	1.21	69.8

MSLV: Mean shoot length value, SD: Standart Deviation, CV: Coefficient of variation, FW: Fresh weight, FWR: Fresh weight reduction, DW: Dry weight, DWR: Dry weight reduction

As gamma irradiation doses increased. all growth parameters exhibited a clear and progressive decline. The untreated control (0 Gy) showed the highest vigour. with a mean shoot length of 13.85 cm, a fresh weight of 45.86 g, and a dry weight of 2.95 g (Table 1). At 2 Gy, shoot length decreased to 11.23 cm. accompanied by 12.8% and 3.7% reductions in fresh and dry weight. respectively. At 5 Gy, shoot length was 12.78 cm. while fresh and dry weights declined by 4.0% and 8.5%. A more pronounced inhibitory effect appeared at 15 Gy, where shoot length dropped to 9.62 cm and biomass reductions reached 43.1% (fresh) and 39.0% (dry). The negative association between dose and shoot elongation is clearly depicted in Figure 1.

Figure 1. Effects of different gamma radiation doses (Gy) on shoot length (cm) of tomato plants at 30th days: *in vitro* germinated plantlets

Growth suppression intensified at 20 Gy, with a shoot length of 9.21 cm and decreases of 27.6% in fresh weight and 14.6% in dry weight. A significant threshold of inhibition occurred at 25 Gy: shoot length fell to 6.96 cm, and fresh and dry weights were reduced by 55.1% and 51.5%. At 30 Gy, shoots reached 7.65 cm, and biomass decreased by 45.1% and 37.3%. Higher doses caused even stronger inhibition: at 40 Gy, shoot length declined to 6.12 cm, with 61.8% and 62.7% reductions in fresh and dry weights.

The most severe effects were observed at ≥50 Gy. At 50 Gy, mean shoot length decreased to 4.50 cm, corresponding to 67.7% and 66.1% reductions in fresh and dry biomass. Extreme suppression was recorded at 60 Gy, where shoot length dropped to 4.23 cm and biomass decreased by 84.1% (fresh) and 78.6% (dry). Further reduction occurred at 70 Gy, with a shoot length of 3.27 cm and declines of 91.2% and 85.4%. At the highest dose (80 Gy), shoot length was 3.88 cm, and fresh and dry biomass decreased by 85.3% and 83.4%, respectively (Table 1; Figure 1).

Variation metrics followed a similar dose-dependent pattern. Standard deviation (SD) and coefficient of variation (CV%) values for shoot length, fresh weight (FW), and dry weight (DW) are presented in Table 1. At low doses (2-5 Gy), shoot length ranged between 11.23 and 12.78 cm, with SD values (2.28-2.34) comparable to the control group (SD = 2.14), indicating similar variability. At 15 Gy and above, shoot length and biomass were substantially reduced, accompanied by lower SD values, reflecting more uniform inhibition across replicates.

Linear regression analysis revealed a statistically significant negative correlation between radiation dose and shoot length ($R^2 = 0.8878$; Figure 2).

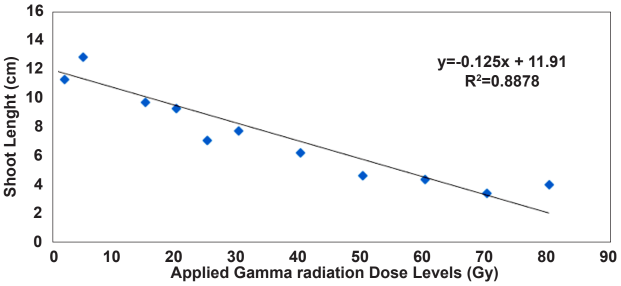

Figure 2. Dose-response linear regression of gamma irradiation (Gy) on shoot length (cm) *in vitro*

The linear regression model:

$$y= -0.125x+11.91(R^2= 0.8878) \qquad (2)$$

effectively described the dose-response trend. Based on this model, the effective mutation dose resulting in a 50% reduction in shoot growth (EMD_{50}) was estimated at 39.8 Gy. A ±10% interval around this value (35.82 - 43.78 Gy) represents a suitable threshold range for inducing genetic variability while maintaining seedling viability.

Fresh weight exhibited a biphasic response to gamma irradiation (Figure 3). FW was highly sensitive at low doses, decreasing by 40-48% at 10 Gy relative to the control. Between 20 and 30 Gy, FW reductions stabilized at 20-30%, indicating a phase of metabolic plateau. Interestingly, at doses above 40 Gy, FW reduction appeared to decrease (5-15%), likely due to a drastic decrease in metabolically active tissue, resulting in fewer measurable stress-related changes. This pattern is consistent with the pronounced decline in shoot elongation shown in Figure 1 and regression outcomes in Figure 3.

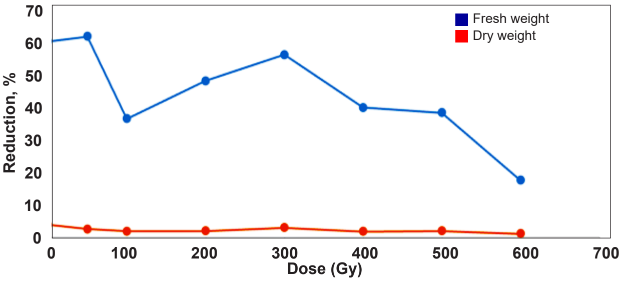

Figure 3. Dose-response *in vitro* plants fresh and dry weight reduction %

In contrast, DW remained relatively stable across the radiation gradient, with minimal reductions (0-3%), indicating that structural biomass accumulation was largely unaffected, whereas water-related metabolic processes (reflected in FW) were more sensitive. These observations correspond closely with the CV% trends reported in Table 1, where DW variation remained low even at higher doses.

In the second phase of the study, tomato seeds were exposed to seven gamma radiation doses ranging from 0 to 600 Gy and cultivated under *ex vitro* conditions. Representative seedling morphology for these treatments is shown in Figure 4.

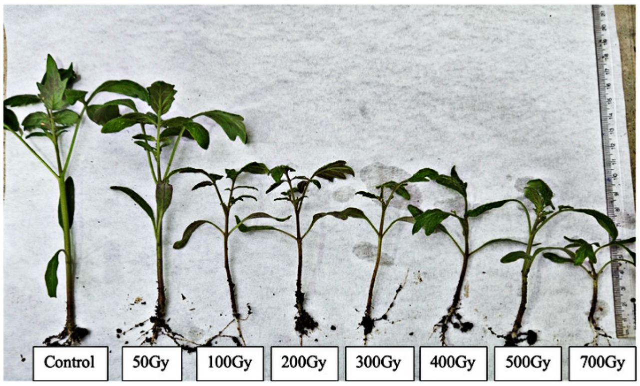

Figure 4. Effects of different gamma radiation doses (Gy) on shoot length (cm) of tomato plants at 30th days: *ex vitro* germinated seedling

Shoot length measurements were used to generate the dose-response regression model (Figure 5), which demonstrated a negative correlation between radiation dose and shoot elongation.

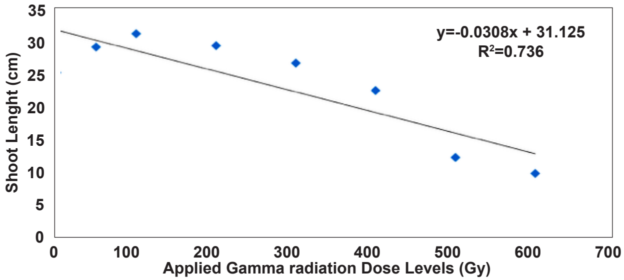

Figure 5. Dose-response linear regression of gamma irradiation (Gy) on shoot length (cm) *ex vitro* tomato for mutation induction

The control group (0 Gy) exhibited a mean shoot length of 24.76 cm, a fresh weight (FW) of 60.3 g, and a dry weight (DW) of 4.0 g (Table 1). At 50 Gy, shoot length increased to 28.56 cm, with a slight FW reduction (−2.5%) and a 33.8% reduction in DW. At 100 Gy, shoot length further increased to 30.59 cm, while FW and DW decreased by 39.5% and 51.5%, respectively. Between 100 and 300 Gy, growth responses remained variable, with shoot lengths ranging from 26.2 to 30.59 cm and moderate biomass reductions.

Pronounced growth inhibition became apparent at 400 Gy, where shoot length decreased to 22.09 cm, accompanied by reductions of 33.7% (FW) and 53.8% (DW). At 500 Gy, shoot length sharply declined to 12.08 cm, with FW and DW reductions of 36.4% and 49.3%, respectively. The most severe suppression occurred at 600 Gy, with shoot length of 9.75 cm and FW/DW reductions of 70.8% and 69.8% (Figure 6).

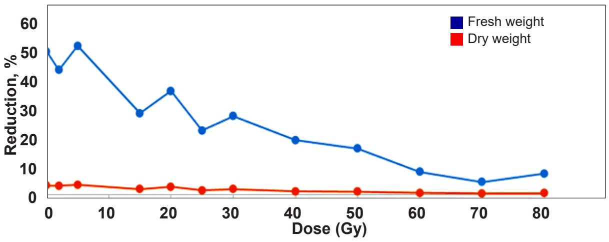

Figure 6. Dose-response *ex vitro* plants fresh and dry weight reduction %

Seedlings grown *ex vitro* generally exhibited higher numerical values for vegetative growth parameters compared with *in vitro* plantlets. The control group showed a mean shoot length of 24.76 cm and a higher SD (7.53), reflecting increased natural variability. At 50 Gy, shoot length reached 28.56 cm and FW increased slightly to 61.8 g, with SD values comparable to the control (Table 1).

Variability in shoot length and biomass was greater under ex vitro conditions, as indicated by higher SD and coefficient of variation (CV%) values (Table 1). Seedlings exposed to 50-300 Gy exhibited considerable natural variation, typical of ex vitro environments, whereas variability decreased at higher doses (400-600 Gy) due to the uniform suppressive effect of gamma irradiation. Thus, high-dose treatments produced more consistent inhibition across replicates.

The regression model for *ex vitro* shoot length (Figure 5) demonstrated a moderate but significant negative relationship:

$$y = -0.0308x + 31.125 (R^2 = 0.736) \quad (3)$$

The regression analysis yielded an R² of 0.736 (Figure 5), indicating a substantial dose-dependent effect on shoot elongation. Based on this model, the effective mutation dose (EMD_{50}) under *ex vitro* conditions was calculated as 608.6 Gy. Doses 10% below (521.1 Gy) and above (636.9 Gy) the EMD_{50} can target different levels of genotypic and cellular sensitivity. This range defines a critical threshold in which genetic variation can be effectively induced while maintaining plant viability.

Ex vitro biomass responses differed from those observed *in vitro*. Fresh weight (FW) declined moderately at low-to-medium doses (10-20% reduction up to 200 Gy) and showed more pronounced decreases at higher doses (25-40% reduction at

400-600 Gy; Figure 6). These results suggest that *ex vitro* plants, supported by soil nutrient availability, microbial interactions, and intact root systems, are more resilient to oxidative and metabolic disruptions. Dry weight (DW) responses remained minimal (1-4% variation), like *in vitro* plants, confirming that structural tissue integrity is largely preserved even under substantial radiation exposure.

3.2 Discussion

This study aimed to evaluate the effects of gamma irradiation on early vegetative development in tomato seedlings under different cultivation environments, to determine optimal mutagen doses for mutation breeding. Understanding the radiosensitivity of tomato (*Solanum lycopersicum* L. var. *cerasiforme*) seeds is essential for effective mutation breeding. In this study, the moisture content of the seeds was measured prior to irradiation, yielding 53.3 % (based on fresh and dry weights of 3 g and 1.4 g, respectively), consistent with values reported for mature tomato seeds. This confirms the seeds' suitability for subsequent gamma irradiation experiments.

The present study examined the effects of gamma irradiation on early vegetative development in *Solanum lycopersicum* L. var. *cerasiforme* under both *in vitro* and *ex vitro* conditions, revealing that the cultivation environment profoundly influences radiosensitivity, growth responses, and phenotypic variability. Specifically, *in vitro* shoot cultures showed pronounced growth inhibition in response to increasing gamma radiation, with an EMD_{50} of approximately 39.8 Gy. In contrast, *ex vitro* seedlings demonstrated much greater tolerance, with an EMD_{50} close to 608.6 Gy. These findings align with and extend previous mutation breeding studies in tomato and related crops, reinforcing the necessity of environment-specific dose calibration when designing mutagenesis protocols. Similar dose-dependent suppression trends have been reported in seed- and tissue-based irradiation studies in tomato, where sensitivity and the effective mutagenic dose vary substantially across genotypes and tissue types [34,35].

Under *in vitro* conditions, shoot length and biomass (both fresh and dry) declined linearly with increasing gamma dose. The derived EMD_{50} value (39.8 Gy) corresponds well with the effective dose ranges reported in earlier tissue-culture-based mutagenesis work. For example, studies on tomato and other Solanaceae using gamma radiation or other physical mutagens often identify optimum mutagenic windows around 25-45 Gy for *in vitro* tissues (e.g., those cultured as explants or calli). The increased radiosensitivity observed *in vitro* is likely attributable to the absence of environmental buffering mechanisms. Specifically, *in vitro* tissues do not possess soil-associated microbial symbionts, exhibit restricted antioxidant defence systems, and depend exclusively on culture media for stress mitigation [29, 36]. According to studies on radiation mutation breeding, gamma rays damage DNA by causing double strand breaks and producing reactive oxygen species (ROS) through water radiolysis. This disruption interferes with cell division and protein production, ultimately resulting in stunted growth and abnormal development. [37-39]. Within this framework, the sharp decline in growth beyond 39.8 Gy indicates that the regenerative capacity of *in vitro* tissues is rapidly overwhelmed by radiation stress, compromising viability and morphogenesis. Quantitative analysis confirmed this trend. Mean values, standard deviations (SD), and coefficients of variation (CV%) for all treatments are summarized in Table 1. A significant increase in variation was observed at higher doses: CV%, which ranged between 6-12% in control and 10 Gy treatments, rose to >30% at 80-100 Gy. This increase reflects the heterogeneous cellular response to DNA damage characteristic of mutagenic stress. Comparable patterns of increased phenotypic variability at higher doses have also been recorded in *in vitro* radiosensitivity tests in tomato regeneration systems [40].

Conversely, *ex vitro*-grown seedlings exhibited much greater tolerance. In some cases, low to moderate doses (e.g., 50-100 Gy) even stimulated growth - a phenomenon consistent with low-dose hormesis previously documented in tomato irradiation studies [35]. Growth remained relatively stable up to intermediate doses (300 Gy), and only at high doses (≥ 400 Gy) did suppression become evident.

The estimated *ex vitro* EMD_{50} value (608.6 Gy) is considerably higher than that observed *in vitro*. This difference can be attributed to the more complex environmental and physiological conditions experienced by *ex vitro*-grown plants. The soil environment provides natural buffering mechanisms, including root-microbe interactions and effective regulation of water and nutrient uptake, which mitigate radiation-induced stress. Additionally, *ex vitro* plants possess fully functional photosynthetic machinery, stomatal regulation, and robust antioxidant defence systems, enabling them to better neutralize reactive oxygen species generated by gamma irradiation [41, 42]. In contrast, *in vitro* tissues, grown under sterile and limited nutrient conditions, lack these compensatory and protective mechanisms, rendering them more sensitive to radiation. Moreover, *ex vitro* plants may exhibit low-dose stimulation (hormesis), a response rarely observed in *in vitro* cultures. Collectively, these factors explain the substantial difference in EMD_{50} values between *in vitro* and *ex vitro* systems.

Phenotypic variability (coefficient of variation, CV%) also followed different trajectories in the two systems. In vitro, CV increased steeply at higher doses, consistent with uniform stress-driven suppression and minimal capacity for compensation [43, 44]. In *ex vitro* conditions, variability remained low at mild and intermediate doses but rose at high doses, reflecting heterogeneous individual responses - likely due to genotypic differences in repair capacity, antioxidant responses, or micro-environmental variation [45, 46]. This emergence of variability under high-dose *ex vitro* irradiation may be especially valuable for mutation breeding, as a broad phenotypic spectrum increases the chances of obtaining desirable mutants[47]. Research on seed irradiation has also shown that increasing dosages can elevate phenotypic variation and alter the relationships between different traits [45]. and field evaluations of irradiated tomato populations [43].

Overall, the present results coincide with classical and recent accounts of gamma-irradiation mutagenesis in tomato. Reviews highlight that gamma-rays are a key method for creating genome-wide variation in mutation breeding, as they induce large chromosomal changes that can reveal traits not easily found through conventional breeding or gene editing [46, 47]. Accordingly, the relatively low *in vitro* EMD_{50} (39.8 Gy) supports the use of tissue culture for rapid generation of mutant variants, while the high *ex vitro* EMD_{50} (608.06 Gy) indicates that seed or soil-grown irradiation gives better survival and phenotypic viability, however, this requires greater doses and careful dose calibration [43-45]. This is consistent with the findings of Chong et al. (2023), who reported that *in vitro* tissues exhibit higher radiosensitivity and require substantially lower doses to reach LD_{50}/EMD thresholds compared with seed-based irradiated systems.

Nevertheless, morphological parameters alone (shoot length, fresh/dry weight) are insufficient to verify stable and heritable mutations. Current mutation-breeding programs are advised to integrate molecular analyses such as TILLING, SNP genotyping, or whole-genome sequencing alongside phenotypic screening, as recommended in the literature. This approach facilitates the detection of stable genetic alterations and minimises the risk of chimerism, particularly when high-dose irradiation is utilised [46, 47]. In addition, epigenetic effects and stress-induced gene expression changes (e.g., antioxidant or repair pathway activation) should be considered, because they may contribute to phenotypic variation in ways that are not strictly genetic, but still relevant for stress-tolerance or other agronomic traits [48].

4. Conclusion

This study demonstrates that the growth environment plays a critical role in determining the radiosensitivity, phenotypic variability, and effective mutation dose (EMD_{50}) of cherry tomato seedlings subjected to gamma irradiation. In vitro plantlets showed pronounced sensitivity, with a consistent reduction in shoot length, fresh weight, and dry weight, resulting in an EMD_{50} of 39,8 Gy. Elevated CV values at increasing doses indicate that *in vitro* tissues amplify radiation-induced variability due to limited physiological buffering. In contrast, *ex vitro* seedlings exhibited substantial tolerance, maintaining growth under moderate-high doses and producing an EMD_{50} of 608.06 Gy, reflecting the protective capacity of soil-based physiological and microbial interactions.

The markedly different EMD_{50} values underscore the importance of tailoring irradiation protocols to the developmental and environmental context of plant material. Based on integrated growth and variability assessments, 35-45 Gy is recommended for *in vitro* shoots, while 520-640 Gy is suitable for *ex vitro* seedlings to achieve sufficient variability without compromising plant viability. These findings provide a practical framework for optimizing gamma-irradiation-based mutagenesis in cherry tomato and highlight the need to complement morphological assessments with molecular analyses in future research to ensure the efficiency and stability of induced mutations.

This study provides a strong empirical basis for developing environment-specific mutagenesis strategies in cherry tomato, underscoring the significant influence of cultivation context on radiosensitivity, variability, and the determination of effective mutation doses.

Author Contributions Statement

Dr. Kadriye Yaprak Kantoğlu: Project design, manuscript editing.
Irmak Çakın: Experimental work, data analysis, manuscript writing.
Assoc. Prof. Dr. Erhan Aksu: Irradiation procedures.

Acknowledgments

This research was supported by the Nuclear Energy Research Institute of the Turkish Energy, Nuclear and Mineral Research Agency (TENMAK NÜKEN). The authors express their sincere gratitude to the TENMAK NÜKEN authorities for their valuable support, and to all members of the agricultural and food research group for their assistance throughout the study.

References

[1] Çelik, Ö., Ayan, A., Meriç, S., & Atak, Ç. (2021). Comparison of tolerance related proteomic profiles of two drought tolerant tomato mutants improved by gamma radiation. *Journal of Biotechnology*, *330*, 35-44. https://doi.org/10.1016/j.jbiotec.2021.02.012.

[2] Klunklin, W., & Savage, G. P. (2017). Effect of gamma irradiation on the physical and chemical properties of cherry tomatoes. *Food Chemistry*, *229*, 417-424. https://doi.org/10.1016/j.foodchem.2017.02.099.

[3] Paduchuri, R., Reddy, K. R., & Reddy, C. S. (2010). Gamma irradiation induced genetic variability in tomato (*Solanum lycopersicum* L.). *Journal of Plant Breeding and Crop Science*, *2*(5), 89-92.

[4] Story, R. N., Smith, R. H., & Jones, D. R. (2010). Influence of gamma irradiation on the shelf life and quality of tomatoes. *Postharvest Biology and Technology*, *56*(1),

74-78.

[5] Food and Agriculture Organization of the United Nations. (2023). *The State of Food and Agriculture 2023: Revealing the true cost of food to transform agrifood systems*. FAO. https://www.fao.org/3/cc7724en/online/cc7724en.html

[6] T.C. Tarım ve Orman Bakanlığı, Tarımsal Ekonomi ve Politika Geliştirme Enstitüsü (TEPGE). (2025). Domates Ürün Raporu 2024 (TEPGE Yayın No. 396, s. 30). https://arastirma.tarimorman.gov.tr/tepge/

[7] Giuliano, G. (2014). Plant carotenoids: Genomics meets multi-gene engineering. *Current Opinion in Plant Biology*, *19*, 111-117. https://doi.org/10.1016/j.pbi.2014.05.006

[8] Ilić, Z. S., Kapoulas, N., & Šunić, L. (2014). Tomato fruit quality from organic and conventional production. In V. Pilipavicius (Ed.), *Organic agriculture towards sustainability* (pp. 147-169). InTechOpen. https://doi.org/10.5772/58239

[9] Mizil, S. N., Al-Shammary, M. Z., & ElKaaby, E. A. (2023). Employment of *in vitro* and gamma mutation for micropropagation of Golden Sunrise cherry tomato (*Solanum lycopersicum* var. *cerasiforme*). *Journal of Survey in Fisheries Sciences*, *10*(3S), 3233-3244.

[10] Story, E. N., Kopec, R. E., Schwartz, S. J., & Harris, G. K. (2010). An update on the health effects of tomato lycopene. *Annual Review of Food Science and Technology*, *1*(1), 189-210. https://doi.org/10.1146/annurev.food.102308.124120

[11] Hwang, E.-S., & Bowen, P. E. (2002). Can the consumption of tomatoes or lycopene reduce cancer risk? *Integrative Cancer Therapies*, *1*(2), 121-132. https://doi.org/10.1177/153473540200100203

[12] Distefano, G., Caruso, M., La Malfa, S., Gentile, A., & Las Casas, G. (2021). Radiogenetic effects of gamma- and fast neutron irradiation on different ontogenetic stages of the tomato. *Radiation Physics and Chemistry*, *11*(3), 215-223. https://doi.org/10.1016/j.radbot.2021.03.005

[13] Distefano, G., Caruso, M., La Malfa, S., Gentile, A., & Las Casas, G. (2022). Gamma irradiation delays tomato (*Solanum lycopersicum*) ripening by inducing transcriptional changes. *Journal of the Science of Food and Agriculture*, *102*(5), 2345-2354. https://doi.org/10.1002/jsfa.12760

[14] Fonseca, R., Micol-Ponce, R., Ozuna, C. V., Castañeda, L., Capel, C., Fernández-Lozano, A., Ortiz-Atienza, A., Bretones, S., Pérez-Jiménez, J. M., Quevedo-Colmena, A. S., López-Fábregas, J. D., Barragán-Lozano, T., Lebrón, R., Faura, C., Capel, J., Angosto, T., Egea, I., Yuste-Lisbona, F. J., & Lozano, R. (2022). Resilient response to combined heat and drought stress conditions of a tomato germplasm collection, including natural and ethyl methanesulfonate induced variants. *Horticulturae*, *10*(6),552. https://doi.org/10.3390/horticulturae10060552

[15] Chun, J. I., Kim, H., Jo, Y. D., Kim, J. B., & Kang, J. H. (2020). Development of a mutant population of Micro-Tom tomato using gamma-irradiation. *Plant Breeding and Biotechnology*, *8*(4), 307-315.

[16] Sikder, S., Biswas, P., Hazra, P., Akhtar, S., Chattopadhyay, A., Badigannavar, A. M., & D'Souza, S. F. (2013). Induction of mutation in tomato (*Solanum lycopersicum* L.) by gamma irradiation and EMS. *Indian Journal of Genetics and Plant Breeding*, *73*(4), 392-399.

[17] Kantoğlu, K. Y., İç, E., Özmen, D., Bulut, F. Ş., Ergun, E., Kantoğlu, Ö., & Özçoban, M. (2023). Gamma rays induced enhancement in the phytonutrient capacities of tomato (*Solanum lycopersicum* L.). *Frontiers in Horticulture*, *2*, 1190145. https://doi.org/10.3389/fhort.2023.1190145

[18] Sherpa, R., Devadas, R., Bolbhat, S. N., Nikam, T. D., & Penna, S. (2022). Gamma Radiation Induced In-Vitro Mutagenesis and Isolation of Mutants for Early Flowering and Phytomorphological Variations in Dendrobium 'Emma White'. *Plants*, *11*(22), 3168. https://doi.org/10.3390/plants11223168

[19] Nahiyan, A. S. M., Rahman, L., Raiyan, S., Mehraj, H., & Uddin, A. F. M. J. (2014). Selection of EMS-induced tomato variants through TILLING for point mutation. *Bangladesh Research Publication Journal*, *10*(2), 214-222. Access link: https://ssrn.com/abstract=3601452

[20] Matsukura, C., Yamaguchi, I., Inamura, M., Ban, Y., Kobayashi, Y., Yin, Y. G., Saito, T., Kuwata, C., Imanishi, S., & Nishimura, S. (2007). Generation of gamma irradiation-induced mutant lines of the miniature tomato (*Solanum lycopersicum* L.) cultivar 'Micro-Tom'. *Plant Biotechnology*, *24*(1), 39-44. https://doi.org/10.5511/plantbiotechnology.24.39

[21] Till, B. J., Cooper, J., Tai, T. H., Colowit, P., Greene, E. A., Henikoff, S., ... & Comai, L. (2007). Discovery of chemically induced mutations in rice by TILLING. *BMC Plant Biology*, *7*, 19. https://doi.org/10.1186/1471-2229-7-19

[22] Watanabe, S., Mizoguchi, T., Aoki, K., Kubo, Y., Mori, H., Imanishi, S., Yamazaki, Y., Shibata, D., & Ezura, H. (2007). Ethylmethanesulfonate (EMS) mutagenesis of *Solanum lycopersicum* cv. Micro-Tom for large-scale mutant screens. *Plant Biotechnology*, *24*(1), 33-38. https://doi.org/10.5511/plantbiotechnology.24.33

[23] Lee, G. J., Chung, S. J., Park, I. S., Lee, J. S., Kim, J. B., Kim, D. S., & Lee, I. S. (2008). Variation in the phenotypic features and transcripts of color mutants of chrysanthemum (Dendranthema grandiflorum) derived from gamma ray mutagenesis. *Journal of Plant Biology*, *51*(6), 418-423. https://doi.org/10.1007/BF03036162

[24] Chen, L., Duan, L., Sun, M., Yang, Z., Li, H., Hu, K., Yang, H., & Liu, L. (2023). Current trends and insights on EMS mutagenesis application to studies on plant abiotic stress tolerance and development. *Frontiers in Plant Science*, *13*, 1052569. https://doi.org/10.3389/fpls.2022.1052569

[25] Sağel, Z., Tutluer, M. İ., & Peşkircioğlu, H. (2002). *Bitki ıslahında mutasyon ve doku kültürü teknikleri* (Kurs notları). Türkiye Atom Enerjisi Kurumu, ANTHAM Nükleer Tarım Radyobiyoloji Bölümü, Ankara 75p.

[26] Simco, B. A., Kantoğlu, K. Y., & Sağel, Z. (2014). *Mutasyon ıslahı ile geliştirilen domates hatlarının verim ve kalite özellikleri*. Uluslararası Mezopotamya Tarım Kongresi, 22-25 Eylül 2014, Diyarbakır, Türkiye.

[27] Karaca, M. (2018). Yeni nesil bitki ıslahı yöntemleri

(moleküler bitki ıslahı) bazı avantaj ve dezavantajları. *Research Journal of Agricultural Sciences*, *11*(1), 39-49. https://doi.org/10.33462/tabad.41479

[28] Kayın, N., & Turan, F. (2023). Bitki Doku Kültürünün Biyoteknolojik Olarak Kullanımı. *Journal of Agricultural Biotechnology*, *4*(1), 1-10. https://dergipark.org.tr/en/pub/joinabt/issue/78158/1300369

[29] Kodym, A., & Afza, R. (2003). Physical and chemical mutagenesis. In Plant *Mutation Breeding and Biotechnology* (pp. 155-170). FAO/IAEA.

[30] Roy, S., Banerjee, A., & Basu, S. (2021). Mutation breeding in plants: Concept, methods and applications. *Journal of Genetic Engineering and Biotechnology*, *19*(1), 128. https://doi.org/10.1186/s43141-021-00218-1

[31] Franco, M. R., Piotto, F. A., Tulmann-Neto, A., & Nogueira, D. G. (2015). Rapid screening for selection of heavy metal-tolerant plants. *Crop Breeding and Applied Biotechnology*, *15*(2), 88-94. https://doi.org/10.1590/1984-70332015v15n2a16.

[32] International Atomic Energy Agency. (1977). *Manual on mutation breeding* (Technical Report Series No. 119). Vienna: IAEA

[33] International Atomic Energy Agency (IAEA). (2018). *Manual on mutation breeding* (3rd ed.; IAEA TECDOC-1426; IAEA TECDOC-1615). IAEA.

[34] Das, P., Islam, M. M., Kabir, M. H., Islam, M., Islam, S. A. M., Islam, R., Jahan, M. T., Roy, P. K., Halder, R., Roy, P. K., Mamun, A. N. K., & Hossain, M. L. (2021). Study on the effect of γ-irradiation (Co-60) on seed germination and agronomic traits in tomato plants (Lycopersicon esculentum L.). *Notulae Scientia Biologicae*, *13*(4), Article 11061. https://doi.org/10.15835/nsb13411061

[35] Zafar, S. A., Aslam, M., Albaqami, M., Ashraf, A., Hassan, A., Iqbal, J., Maqbool, A., Naeem, M., Al-Yahyai, R., & Tan Kee Zuan, A. (2022). Gamma rays induced genetic variability in tomato (*Solanum lycopersicum* L.) germplasm. *Saudi Journal of Biological Sciences*, *29*(5), 3300-3307. https://doi.org/10.1016/j.sjbs.2022.02.008

[36] Büyükdinc, D. T., Kantoglu, K. Y., Karatas, A., Ipek, A., & Ellialtıoglu, S. S. (2019). Determination of effective mutagen dose for carrot (Daucus carota ssp sativus var atrorubens alef and D. carota) callus culture. *International Journal of Science and Technology Research*, *5*(3), 15-23.

[37] Britt, A. B. (1999). Molecular genetics of DNA repair in higher plants. *Trends in Plant Science*, *4*(1), 20-25. https://doi.org/10.1016/S1360-1385(98)01354-5

[38] Kovács, E., & Keresztes, Á. (2002). Effect of gamma and UV-B/C radiation on plant cells. *Micron*, *33*(2), 199-210. https://doi.org/10.1016/S0968-4328(01)00011-9

[39] De Micco, V., & Aronne, G. (2012). Morpho-anatomical traits for plant adaptation to drought. *Plant Responses to Drought Stress*, 37-61.

[40] Chong, S. P., Ahmad, Z., Bahari, N., & Shamsudin, S. (2023). Radiosensitivity test of *in vitro* regeneration tomato (*Solanum lycopersicum*) in radiation mutation. In *Proceedings of the National Instrumentation and Technology Conference* (NITC) 2023 (Abstract No. PG-07). Nuklear Malaysia / NITC.

[41] Yamaguchi, H., Degi, K., & Morishita, T. (2008). Effects of ion beam irradiation on mutation induction and nuclear DNA content in chrysanthemum. *Scientia Horticulturae*, *118*(1), 108-112.https://doi.org/10.1016/j.scienta.2008.05.005

[42] Zaka, H. F., Lyle, J. M., & Kovalchuk, I. (2002). Radiobiological aspects of mutation breeding in plants. *Mutation Research*, *510*(1-2), 141-152. https://doi.org/10.1016/S0027 5107(02)00017-6

[43] Wi, S. G., Chung, B. Y., Kim, J. S., Kim, J. H., Baek, M. H., Lee, J. W., & Kim, Y. S. (2007). Effects of gamma irradiation on morphological changes and biological responses in plants. *Micron*, *38*(6), 553-564. https://doi.org/10.1016/j.micron.2006.11.002

[44] Kim, J. H., Baek, M. H., Chung, B. Y., Wi, S. G., & Kim, J. S. (2004). Alterations in the photosynthetic pigments and antioxidant machineries of red pepper (Capsicum annuum L.) seedlings from gamma-irradiated seeds. *Journal of Plant Biology*, *47*(4), 314-321. https://doi.org/10.1007/BF03030542

[45] Kantoğlu, K. Y., Çakın, I., Çetintaş, A. O., Göktuğ, A., & Laouini, M. (2025). Determining the effective mutation dose and impacts of irradiation for some vegetable species. *Agriculture and Forestry*, *71*(2), 7-20. https://doi.org/10.17707/AgricultForest.71.2.03

[46] Mba, C., Afza, R., Bado, S., & Shu, Q. Y. (2010). Mutagenic radiations: Molecular mechanisms and applications in crop improvement. In Q. Y. Shu (Ed.), *Induced plant mutations in the genomics era* (pp. 1-17). FAO/IAEA.

[47] Shu, Q. Y., Forster, B. P., & Nakagawa, H. (2012). *Plant mutation breeding and biotechnology*. CABI.

[48] Chinnusamy, V., & Zhu, J. K. (2009). Epigenetic regulation of stress responses in plants. *Current Opinion in Plant Biology*, *12*(2), 133-139. https://doi.org/10.1016/j.pbi.2008.12.006.