PHYSIOLOGICAL AND MORPHOLOGICAL ALTERATIONS IN FENUGREEK (*TRIGONELLA FOENUM-GRAECUM* L.) SEEDS EXPOSED TO GAMMA RADIATION

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Abstract. Mutation breeding was developed to overcome the inherent limitations of conventional plant breeding, which include restricted genetic diversity, protracted selection cycles, and insufficient resistance to diseases and pests. This approach has been widely applied across various crop species using both chemical and physical mutagens. The objective of this research was to investigate the morphological and physiological variations in fenugreek (*Trigonella foenum-graecum* L.) plants resulting from exposure to different doses of gamma rays, with a focus on the effects of physical mutations. For this study, fenugreek seeds were subjected to γ-irradiation using a Co^{60} source at dosage levels of 0, 50, 100, 200, 300, 400, and 500 Gy. On the 35th day after sowing, plant height, root length, fresh and dry weights of shoots and roots, leaf color values, chlorophyll content (chlorophyll a, chlorophyll b, and total chlorophyll), carotenoid content, proline concentration, and the activities of superoxide dismutase (SOD) and catalase (CAT) were evaluated for seedlings derived from each treatment. The findings revealed significant dose-dependent reductions in plant height, root length, root fresh weight, the chlorophyll b* (component of leaf color), as well as all measurements of chlorophyll and carotenoids in seedlings derived from irradiated seeds. On the other hand, both proline accumulation and SOD activity showed a dose-dependent increase, peaking at 500 Gy. Overall, these findings indicate that progressively higher mutagen doses generally compromise seedling growth and physiological performance. Nonetheless, the capacity of γ irradiation to generate phenotypic and physiological variation in fenugreek is evident, underscoring its potential as a tool in future breeding initiatives.

Keywords: *chlorophyll, fenugreek (Trigonella foenum-graecum L.), mutation breeding, proline, γ-irradiation*

Introduction

Medicinal and aromatic plants have been a cornerstone of human health and wellness, serving as remedies for a wide range of ailments throughout history. From the earliest communities, people have relied on the healing properties of plants, a knowledge that was gained through careful observation and practical experience. This accumulated knowledge has been passed down through generations, ensuring the continuity of plant-based therapies in Anatolia's long-standing tradition of folk medicine.

Many pharmaceutical and phytopharmaceutical products used in modern, traditional, and complementary medical practices contain active compounds derived from medicinal and aromatic plants. According to research conducted by the World Health Organization (WHO), approximately 20,000 plant species are utilized worldwide for therapeutic purposes, which highlights the long-standing use of plant-based treatments and emphasizes their potential for even greater effectiveness when supported by contemporary scientific methods (Uğur and Kan, 2016). Among these medicinal and

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aromatic species, fenugreek stands out for its diverse applications in both nutrition and medicine, a fact that will be explored in detail in this article.

Fenugreek (*Trigonella foenum-graecum* L.), a member of the Fabaceae family, derives its generic name *Trigonella* from the Latin for "little triangle" from the yellowish white, triangular flowers characteristic of the species (Flammang et al., 2004). The plant is known by diverse vernacular names: *methi* in Hindi, Urdu, Punjabi, and Marathi; *hulba* in Arabic; *moshoseitaro* in Greek; *uluva* in Malayalam; *shoot* in Hebrew; *dari* in Persian; and "hayseed" in English. Considered one of the oldest medicinal plants in the Fabaceae, fenugreek's historical cultivation, dating back to 4000 BCE, is a testament to its resilience and adaptability in the face of changing environments (Altuntaş et al., 2005).

References to fenugreek, a plant with considerable historical significance and medicinal benefits, can be found in the Ebers Papyrus, one of the oldest surviving medical texts compiled in Egypt around 1500 BCE. Today, fenugreek is cultivated commercially across a diverse range of regions, including India, Pakistan, Afghanistan, Iran, Nepal, Egypt, France, Spain, Türkiye, Morocco, North Africa, the Middle East, and Argentina (Ahmad et al., 2016). There remains some uncertainty regarding the number of species within the genus due to the notable diversity in morphology, growth habits, flower color, leaf and stem structure, and chemical composition (Sarwar et al., 2020). At the same time, some researchers have proposed that the genus may encompass as many as 260 species, but only 18 species are currently recognized. In Türkiye, three distinct cultivars of this species have been officially registered.

Among the released genotypes, the cultivars "Gürarslan," "Berkem," and "Çiftçi" have been formally registered by the Faculty of Agriculture at Ankara University, the Faculty of Agriculture at Dicle University, and the Republic of Türkiye Ministry of Agriculture and Forestry Transitional Zone Agricultural Research Institute, the Transitional Zone Agricultural Research Institute, respectively. According to data from the Turkish Statistical Institute (TÜİK) for 2022, fenugreek is cultivated in several provinces of Türkiye, including Afyonkarahisar, Amasya, Ankara, Karaman, Kastamonu, Kayseri, Konya, Samsun, Sivas, Tokat, Yozgat, and Çorum. The total cultivated area is approximately 8900 decares (about 890 ha), which produced a total of 1040 tons of seed, resulting in an average yield of 117 kg per decare (TÜİK, 2023).

Achieving high productivity per unit area primarily depends on the implementation of appropriate cultural practices and the inherent yield potential of the seed. The potential for enhanced yield capacity in seeds contributes to increased output per unit area. Consequently, it is essential that the initial planting material, specifically seeds, be of consistently high quality, particularly in crops such as fenugreek. In large-scale commercial production, the use of hybrid (F1) seeds is especially advantageous, as they facilitate significantly higher yields per unit area (Kantoğlu, 2014). Hybrid seeds that result from crosses between elite parent plants combine desirable traits, thereby increasing overall productivity. Furthermore, growers tend to favor seeds that are well-suited to local agro-ecological conditions, demonstrate strong performance, and fulfill consumer expectations for quality and appearance. This preference further fuels the demand for such seeds (Akalp and Pirinç, 2024).

Among the strategies plant breeders use to obtain high-quality seed are conventional breeding, tissue culture, anther culture, and various biotechnological approaches. Mutation breeding, a highly efficient technique that can rapidly generate extensive genetic variation, offers breeders additional flexibility (Maurya and Bahadur, 2022; Dongfu et al., 2022). The use of physical mutagens, such as X-rays, gamma rays, and UV

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radiation, as well as chemical mutagens like ethyl methanesulfonate, sodium azide, and diethyl sulfate, allows for rapid mutation breeding. This technique enables the production of plants with desirable traits and is becoming increasingly popular (Gerami et al., 2017). In this study, seeds were irradiated with various doses of gamma rays from a Co^{60} source to assess their effects on germination and early seedling growth. The results aim to provide insights into how radiation treatments influence crop cultivation and help identify the optimal radiation doses for controlled conditions.

Materials and methods

Materials

Plant material

The biological material was comprised of commercial seeds from the fenugreek cultivar "Çiftçi" (*Trigonella foenum-graecum* L.). This cultivar is characterized by an upright growth habit, reaching an average height of 60–80 cm, producing white flowers, and yielding yellowish seeds. In its primary cultivation regions (Central Anatolia and the Transitional Zone), it is typically sown as a spring crop during March and April.

Experimental site

The study was carried out in the laboratories and polycarbonate greenhouses of the Faculty of Agriculture at Dicle University in Diyarbakır, Türkiye. Equipped with an automated climate control system, the greenhouse maintained average daytime and nighttime temperatures of 25°C and 22°C, respectively. Throughout the 45-day duration of the experiment, air circulation and humidity levels were continuously monitored and adjusted to ensure optimal conditions for plant growth and development.

Methods

Gamma irradiation treatment

Fenugreek seeds were irradiated at the Turkish Energy, Nuclear and Mineral Research Agency (TENMAK) using a Co60 γ-ray source at doses of 0, 50, 100, 200, 300, 400, 500, and 600 Gy. Prior to irradiation, the seed lot was mechanically cleaned through sieving and calibrated to ensure experimental uniformity in size, shape, and color. Any seeds exhibiting physical damage or visible abnormalities were discarded. To reduce microbial contamination, the seeds were surface-sterilized by immersion in a 2% (v/v) sodium hypochlorite solution for 5 min, followed by multiple rinses with sterile distilled water.

Growing medium and sowing

The substrate consisted of a mixture of peat and perlite (2:1, v/v) that exhibited high capacity for air and water retention. Irradiated and control (non-irradiated) seeds were sown to a consistent depth in 45-cell plug trays. Immediately following sowing, the trays received light irrigation, while the relative humidity in the greenhouse was maintained at 70–75%. All seeds were sown on the same day to ensure uniformity and eliminate temporal variation during germination and the initial stages of seedling development.

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Experimental design

The experiment was arranged in a factorial design comprising seven γ irradiation doses (0, 50, 100, 200, 300, 400, and 500 Gy) with five replications. Each replication contained 45 plants, providing a broad range of variation for analysis. Throughout the 45-day growth period, the seedlings were provided with regular irrigation, ventilation, and measures for pest and disease control, while environmental conditions such as temperature, relative humidity, and light were maintained as consistently as possible.

Termination of the experiment and data collection

At 45 days after sowing, the seedlings were carefully and gently removed from the plug trays, ensuring the root systems were rinsed under tap water to avoid any potential mechanical damage, and all measurements were taken. Detailed records of seedling growth and morphological traits were compiled at this stage. The additional determinations conducted are outlined below.

Leaf chlorophyll and carotenoid contents (mg/g)

Chlorophyll a, chlorophyll b, total chlorophyll, and carotenoid concentrations were quantified using a spectrophotometer (Hitachi U 1800, Japan) at wavelengths of 663 nm, 645 nm, and 450 nm, respectively. For each treatment, leaves from ten plants were pooled, and 0.25 g of fresh tissue was homogenized with a small quantity of $CaCO_3$ and 15 mL of 80% (v/v) acetone. The homogenate was brought to a final volume of 20 mL with acetone and centrifuged at 4°C and 6000 rpm for 5 min. Subsequently, 4 mL of the resulting supernatant was transferred to a fresh tube and adjusted to 16 mL with acetone. Finally, absorbance was measured, and pigment concentrations (chlorophyll a, chlorophyll b, total chlorophyll, and carotenoids) were quantified on a fresh-weight basis (mg/g) using the equations of Strain and Svec (1966).

$$\text{Chlorophyll a (Chl a)(mg/g)} = [\ 11.64(A663) - 2.16(A645)] \quad \text{(Eq.1)}$$

$$\text{Chlorophyll b (Chl b)(mg/g)} = [20.97(A645) - 3.94(A663)] \quad \text{(Eq.2)}$$

$$\text{Total Chlorophyll (mg/g)} = \text{Chl a} + \text{Chl b} \quad \text{(Eq.3)}$$

$$\text{Carotenoid (mg/g)} = [1000(A450) - 2.27\ (\text{Chl a}) - 81.4(\text{Chl b})]/227 \quad \text{(Eq.4)}$$

Proline content (μg/g)

At the seedling stage, following the removal of plants from the trays and a gentle washing of their roots, proline analysis was performed. For each treatment, leaves from ten plants were sampled. The quantification of free proline was conducted according to the protocol established by Bates et al. (1973). A total of 0.5 g of fresh leaf tissue from each plant was ground in a porcelain mortar with liquid nitrogen. The resulting pulverized tissue was then homogenized in 3% (w/v) sulfosalicylic acid and filtered through Whatman No. 1 paper. The need for precision in these steps cannot be overstated, as it directly impacts the quality of the filtrate and the accuracy of the proline concentration measurement. The filtrate was then combined with acid ninhydrin and glacial acetic acid, and subsequently incubated in a water bath at 100°C for 1 h. To stop the reaction, the

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tubes were placed in an ice bath, and the chromophore was extracted with toluene. The absorbance of the toluene phase was measured at 520 nm using a spectrophotometer, with the proline concentration expressed as μg/g of fresh weight.

Superoxide dismutase (SOD) activity (EU/g)

SOD activity was assessed spectrophotometrically following the protocol established by Agarwal and Pandey (2004). The reaction mixture, totaling 3 mL, contained 13 mM L-methionine, 63 μM nitro-blue tetrazolium (NBT), 50 mM KH_2PO_4 buffer (pH 7.8), 0.1 mM EDTA, and 13 μM riboflavin. To initiate the assay, 2.58 mL of this reaction mixture was transferred to a 3 mL quartz cuvette. Following this, 30 μL of enzyme extract was added, and the volume was brought to 3 mL by incorporating 390 μL of the 13 μM riboflavin solution. The cuvette was exposed to a white-light source for 15 min, after which the light was turned off to cease the reaction. The reduction of NBT was measured at 560 nm against a blank using a spectrophotometer (Shimadzu UV-1800, Japan). One enzyme unit (EU) was defined as the amount of enzyme required to reduce NBT by 50% under the specified assay conditions, and the activities were expressed as EU per gram of fresh weight. For each treatment, SOD activity was evaluated on 12 seedlings, comprising four plants per replicate.

Catalase (CAT) activity (EU/g)

Catalase activity was assessed using the method established by Gong et al. (2001), which monitors the reduction in absorbance at 240 nm as hydrogen peroxide (H_2O_2) is decomposed into oxygen (O_2) and water (H_2O). To prepare the assay, 1.475 mL of 103 mM KH_2PO_4 buffer (pH ≈ 7.0) and 1.5 mL of 40 mM H_2O_2 were combined in a 3 mL quartz cuvette. Following this, 25 μL of the enzyme extract was introduced to initiate the reaction. The change in absorbance at 240 nm was recorded using a Shimadzu UV-1800 spectrophotometer (Japan), and the rate of decrease in absorbance per minute was calculated to determine catalase activity. The results are presented as enzyme units per gram of fresh weight (EU/g). The catalase activity of each treatment was evaluated using 12 seedlings (four plants per replicate).

Plant height (cm): At the M1–M2 transplanting stage (and, subsequently, on field-established plants), shoot length was measured with a ruler on 30 plants per irradiation dose.

Root length (cm): After the formation of four true leaves and a stem diameter approximating that of a pencil, root length was recorded with a ruler on 30 seedlings per dose.

Shoot fresh weight (g): Shoot fresh weight (g): For each dose, the shoots (leaves + stems) of 30 seedlings were excised at the root–shoot junction, blotted, and weighed immediately on an analytical balance.

Shoot dry weight (g): The same shoot samples were oven dried at 70°C for 24 h and re-weighed to determine dry biomass.

Root fresh weight (g): Roots from the same 30 seedlings were weighed fresh on an analytical balance.

Root dry weight (g): Roots were subsequently oven dried at 70°C for 24 h and re-weighed.

Color parameters (l, a*, b*):* Leaf color was measured on 30 plants per treatment with a portable color meter (Konica Minolta CM 3220d, Japan; 2005) following

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McGuire's (1992) protocol. Increasing a* and b* values denote greater chromatic saturation or color vividness (Keskin et al., 2017). In the CIELAB system:

l* represents lightness, ranging from 0 (black) to 100 (white);

a* denotes the red–green axis, extending from -60 (green) to + 60 (red);

b* denotes the yellow–blue axis, extending from −60 (blue) to + 60 (yellow).

Statistical analysis of the data

Data were analyzed using the statistical software package JMP (SAS Institute, 2007). An analysis of variance (ANOVA) was conducted to evaluate mean differences among treatment groups, applying Tukey's HSD multiple-comparison test with a significance level set at $p < 0.05$. This method enabled a statistical examination of the effects of various γ-irradiation doses on plant development.

Results and discussion

Physiological and morphological data derived from exposing *Trigonella foenum-graecum* L. seeds to graded doses of Co^{60} γ radiation were analyzed and statistically evaluated ($p < 0.05$). The results show that γ-irradiation produced dose-dependent effects on the growth parameters of fenugreek (*Table 1*).

Shoot and root length

Shoot elongation was greatest in the non-irradiated control group, averaging 13.0 cm. In contrast, exposure to the highest gamma-ray dose of 500 Gy reduced the mean shoot length to 8.50 cm, which was the shortest value recorded. The analysis of root length values yielded consistent findings. The control group demonstrated the greatest root length at 6.11 cm, whereas treatments applied at 50 Gy and above resulted in a significant reduction in root length. These findings indicate that high doses of gamma irradiation significantly inhibit meristematic activity, thereby reducing both shoot and root development in fenugreek. Similar findings were reported by Gerami et al. (2017) in chickpea (*Cicer arietinum* L.), where shoot length exhibited a progressive decrease with increasing irradiation doses. Numerous other studies, especially those focusing on pepper and various horticultural crops, also document a reduction in shoot height following exposure to physical or chemical mutagens (Kantoğlu et al., 2014; Pharmawati et al., 2018; Jabeen and Mirza, 2002; Dongfu et al., 2022; Akalp and Pirinç, 2024). In their investigation of common beans, Undirwade and Kulkarni (2019) analyzed seedling and plant height in seeds treated with varying concentrations of chemical mutagens ethyl methanesulfonate (EMS) at 0.05%, 0.10%, and 0.15%, and sodium azide (SA) at 0.010%, 0.015%, and 0.020%, as well as with gamma irradiation doses of 5, 10, and 15 kR. It has been observed that maximum seedling and plant heights were recorded in the control and 0.05 % EMS treatments; as the mutagen dose increased, both traits exhibited a dose-dependent reduction.

Fresh and dry weights

Increasing the dose of γ-rays did not significantly affect the fresh weight of shoots ($p > 0.05$). In contrast, in terms of root fresh weight, the plants treated with 50 Gy exhibited statistically higher values, averaging 0.21 g, compared to the other groups. In contrast, the control group and the plants treated with 500 Gy displayed the lowest root

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fresh weights. The root fresh weight of these groups followed a similar trend to that of the plants treated with 100, 200, 300, and 400 Gy. Similar trends were observed by Akalp and Pirinç (2025) in pepper plants, where increasing concentrations of EMS resulted in dose-dependent decreases in both fresh and dry weights compared to the control.

Leaf color pigments

In the current study, the effects of different γ-irradiation doses on L* and a* color parameters of fenugreek leaves were not statistically significant ($p > 0.05$); whereas treatment differences were significant for b* ($p = 0.0134$). The highest values were recorded at 400 Gy (52.57); however, with the exception of the 200 Gy group, exhibited similar statistical results. (*Table 1*). In a related study utilizing EMS mutagenesis in peppers, Cheng et al. (2019) identified leaf color mutants and conducted genetic and physicochemical characterization of the altered pigmentation, highlighting the sensitivity of leaf color traits to mutagenic treatments. Elevated mutagen doses have been shown to disrupt leaf chromatic attributes and impair photosynthetic performance, resulting in plants with altered foliar pigmentation. Elevated mutagen doses have been shown to disrupt leaf chromatic attributes and impair photosynthetic performance, resulting in plants with altered foliar pigmentation. Siddique et al. (2000) likewise documented that several distinct categories of mutation markedly affect fruit morphology and color. Consistent with these observations, Akalp and Pirinç (2025) reported that progressively higher exposures to physical mutagens, specifically γ irradiation and UV radiation, induced pronounced, dose-dependent reductions in the CIELAB color coordinates l*, a*, and b* in pepper.

Chlorophyll and carotenoid contents

The findings indicate that increasing doses of γ-irradiation lead to a significant decrease in the concentrations of chlorophyll a (*Eq. 1*), chlorophyll b (*Eq. 2*), and total chlorophyll (*Eq. 3*) in fenugreek leaves.

Chlorophyll a achieved its peak concentration in the control samples, measuring 5.75 µg/mg, while the lowest concentration was recorded at the 500 Gy dose, at 3.79 µg/mg. Similarly, chlorophyll b exhibited its highest values in the control (4.33 µg/mg), at 50 Gy (4.37 µg/mg), and 200 Gy (4.46 µg/mg). However, there was a significant decline in chlorophyll b at the 400 Gy dose (3.03 µg/mg), which further dropped to 2.68 µg/mg at the 500 Gy dose. Total chlorophyll content also decreased significantly, falling from 10.58 µg/mg in the samples of the control group to 6.54 µg mg at the 500 Gy dose ($p < 0.001$). Carotenoid (*Eq. 4*) levels exhibited a similar dose-dependent reduction (*Table 1*).

These findings further suggest that γ-irradiation has an adverse effect on the structure and function of chloroplasts. Analysis of carotenoid levels showed that control plants had the highest concentration at 4.82 µg/mg, while the lowest concentrations were recorded at 300 Gy (3.04 µg/mg) and 500 Gy (3.03 µg/mg). Consistent with this interpretation, Dongfu et al. (2022) reported that exposure to γ-rays leads to chlorophyll degradation in Arabidopsis species.

The reduction in carotenoid content is likely attributable to the suppression of antioxidant defense mechanisms that typically protect the photosynthetic apparatus against light-induced damage (Ahmad et al., 2016). Examining the effects of various

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EMS concentrations (0.2, 0.3, and 0.4 %) on chlorophyll and ascorbic-acid levels in pepper, Soyam (2021) found that chlorophyll a peaked at the highest EMS dose (0.4 %), whereas chlorophyll b was greater in the control group and 0.2 % EMS treatments; total chlorophyll likewise reached its maximum at 0.4 % EMS. In tomato, Dutta et al. (2017) reported that leaves of an M_4 mutant line contained an average total chlorophyll concentration of 318.52 mg/100 g fresh weight, compared with 198.25 mg/100 g in the wild-type control group. Collectively, these studies highlight that modest increases in mutagen dosage can, under certain conditions, enhance chlorophyll accumulation. Dongfu et al. (2022) reported a variety of mutant phenotypes observed at the M1 stage, specifically during the seedling, flowering, and maturity phases. At the seedling stage, it was noted that leaf-shape mutations resulted in leaves that appeared to consist of two distinct laminae, each possessing its midrib and apex. Furthermore, alterations in leaf color and morphology were documented; leaves exhibited chlorosis, becoming completely white or yellow, whitish-green, or displaying rough and matte surfaces. In a related study on tomato, Dutta et al. (2017) demonstrated that moderate increases in mutagen dosage significantly raised the mean total carotenoid content in the leaves of M4 mutants.

Proline accumulation

Proline is widely recognized as a key biochemical marker of abiotic stress in plants, playing a vital role in osmotic adjustment and cellular protection. In this study, leaf proline content showed a significant increase with higher doses of γ-irradiation ($p < 0.001$). The concentration rose from 0.32 μmol/g of fresh weight in the control group to 1.26 μmol/g at a dose of 500 Gy. indicating that γ-irradiation induces osmotic stress, leading to an accumulation of proline. Similarly, Maurya and Bahadur (2022) reported that mutation-induced stress elevated proline levels, and Akalp and Pirinç (2025) also observed a dose-dependent increase in pepper, with the highest accumulation recorded at 500 Gy.

SOD and CAT activity

The analysis of antioxidant enzyme activities demonstrated a dose-dependent response for superoxide dismutase (SOD) activity at an irradiation dose of 500 Gy, attaining a measure of 1.06 EU/g of fresh weight. In comparison, the other irradiation doses and the control group were found to belong to a single statistical group, exhibiting significantly lower SOD activity values. In contrast, CAT activity did not vary significantly among irradiation doses. A similar study by Akalp and Pirinç (2025) reported a dose-dependent increase in both SOD and CAT activities in pepper, with peak levels observed at 500 Gy. Furthermore, Dongfu et al. (2022) found that SOD and CAT activities were lowest in control and low-dose seedlings but increased markedly at higher gamma irradiation levels. This study demonstrates that γ-irradiation leads to significant, dose-dependent changes in both the morphological and physiological characteristics of fenugreek. The results related to plant height revealed that the shortest plants were found in the group treated with 500 Gy. Additionally, plants treated with 100 Gy, 200 Gy, 300 Gy, and 400 Gy exhibited similarly reduced height values. In contrast, both the control group and the plants treated with 50 Gy had greater heights (*Table 1*). Therefore, the dosage range of 100 to 500 Gy appears to be the most effective for inducing beneficial variations in *Trigonella foenum-graecum* L. for breeding applications.

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Table 1. *Physiological and morphological changes observed after gamma radiation application at different doses to fenugreek*

Doses (Gy)	Plant height (cm)	Root length (cm)	Shoot fresh weight (g)	Shoot dry weight (g)	Root fresh weight (g)	Root dry weight (g)	l*	a*	b*
Control	13.10 ± 0.58 a	6.11 ± 0.34 a	0.30 ± 0.09 a	0.01 ± 0.00 a	0.13 ± 0.01 b	0.00 ± 0.00 a	49.78 ± 2.53 a	-19.02 ± 0.46 a	42.92 ± 2.44 ab
50	10.54 ± 0.41 b	4.87 ± 0.37 ab	0.17 ± 0.02 a	0.01 ± 0.00 a	0.21 ± 0.01 a	0.00 ± 0.00 a	52.37 ± 1.72 a	-18.95 ± 1.18 a	43.25 ± 1.79 ab
100	9.71 ± 0.36 bc	3.79 ± 0.30 bc	0.16 ± 0.01 a	0.01 ± 0.00 a	0.19 ± 0.02 ab	0.00 ± 0.00 a	48.21 ± 2.67 a	-18.55 ± 0.71 a	45.07 ± 1.68 ab
200	9.19 ± 0.23 bc	3.32 ± 0.23 cd	0.15 ± 0.01 a	0.01 ± 0.00 a	0.19 ± 0.01 ab	0.00 ± 0.00 a	44.19 ± 2.05 a	-17.82 ± 1.35 a	33.23 ± 3.59 b
300	9.21 ± 0.29 bc	3.15 ± 0.26 cd	0.16 ± 0.02 a	0.01 ± 0.00 a	0.17 ± 0.02 ab	0.00 ± 0.00 a	48.43 ± 2.41 a	-20.56 ± 0.61 a	41.71 ± 2.93 ab
400	9.29 ± 0.34 bc	2.97 ± 0.17 cd	0.14 ± 0.02 a	0.01 ± 0.00 a	0.16 ± 0.02 ab	0.00 ± 0.00 a	42.86 ± 3.75 a	-19.11 ± 1.37 a	52.57 ± 4.15 a
500	8.50 ± 0.32 c	2.40 ± 0.25 d	0.15 ± 0.02 a	0.01 ± 0.00 a	0.13 ± 0.02 b	0.00 ± 0.00 a	43.27 ± 3.72 a	-16.78 ± 1.66 a	38.47 ± 4.47 ab
CV	3.44	25.29906542	72.93958398	21.43666226	29.94941834	48.17610063	20.26064712	-20.64286976	25.55320638
LSD	1.679	2.75	0.24	0.004	0.101	0.001	17.102	6.749	20.869
P value	0.002	<.0001	0.0929	0.5183	0.0033	0.5087	0.2124	0.4819	0.0134

Table 1. *Continuation*

Doses (Gy)	Chlorophyll a (µg/mg)	Chlorophyll b (µg/mg)	Total chlorophyll (µg/mg)	Carotenoid (µg/mg)	Proline (µmol/g)	SOD (EU/g)	CAT (EU/g)
Control	5.75 ± 0.23 a	4.33 ± 0.29 a	10.58 ± 0.23 a	4.82 ± 0.30 a	0.32 ± 0.02 d	0.84 ± 0.03 b	0.82 ± 0.08 a
50	5.15 ± 0.28 ab	4.37 ± 0.20 a	9.73 ± 0.21 ab	4.28 ± 0.24 ab	0.37 ± 0.02 cd	0.83 ± 0.01 b	0.82 ± 0.07 a
100	5.29 ± 0.20 ab	4.06 ± 0.21 ab	8.93 ± 0.33 b	4.09 ± 0.28 abc	0.38 ± 0.02 cd	0.83 ± 0.01 b	0.83 ± 0.07 a
200	5.35 ± 0.31 a	4.46 ± 0.22 a	8.77 ± 0.28 b	3.56 ± 0.16 bc	0.61 ± 0.06 bc	0.86 ± 0.01 b	1.49 ± 0.41 a
300	4.72 ± 0.22 abc	3.35 ± 0.15 bc	8.54 ± 0.28 b	3.04 ± 0.28 c	0.65 ± 0.05 b	0.83 ± 0.01 b	0.83 ± 0.07 a
400	4.13 ± 0.24 bc	3.03 ± 0.09 c	7.10 ± 0.34 c	3.12 ± 0.25 bc	0.85 ± 0.08 b	0.85 ± 0.01 b	0.86 ± 0.07 a
500	3.79 ± 0.24 c	2.68 ± 0.11 c	6.54 ± 0.27 c	3.03 ± 0.20 c	1.26 ± 0.09 a	1.06 ± 0.07 a	1.10 ± 0.07 a
CV	17.47	1.68	11.12	22.88	29.96	7.17	38.08
LSD	1.887	17.59517848	2.938	1.879	0.665	0.15	0.6
P value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.0004	0.1319

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Conclusion

Plant breeding programs around the world, including in Türkiye, initially relied on traditional breeding methods and tissue culture techniques. Over time, these programs have evolved to incorporate advanced biotechnologies. However, the development of novel cultivars via conventional breeding, anther culture, and related biotechnological methods often requires extensive time and labor. Generating new variation within existing genetic backgrounds through short-term efforts is therefore impractical. To overcome this limitation, breeders have increasingly turned to artificial mutagenesis to induce rapid genetic diversity. Accordingly, research into the application of various physical and chemical mutagens for generating novel variation has intensified in recent years (Jenni et al., 2006; Kökpınar et al., 2021).

Mutations contribute to the generation of novel variation by inducing abrupt, heritable alterations in plant chromosomes or genes. Accordingly, this study applied Co60-derived γ-irradiation as a physical mutagen to fenugreek (*Trigonella foenum-graecum* L.) to develop lines possessing enhanced physiological and morphological traits, elevated market value, and superior yield potential. Exposure to a broad spectrum of γ-ray doses from seed germination through the seedling stage elicited significant, dose-dependent variation, which was quantified through both morphological assessments and biochemical stress markers. The findings indicate that low-to-moderate γ-radiation doses (50–200 Gy) induce minimal stress while preserving the capacity to generate functional variation in key agronomic traits such as plant height, pigment concentration, and biomass. Hence, this dose range may be considered the optimal threshold for mutation breeding. In contrast, high doses (≥300 Gy) elicited inhibitory effects on root development and the biosynthesis of photosynthetic pigments (chlorophyll a, chlorophyll b, and carotenoids), clearly demonstrating the dose-dependent phytotoxicity of γ-radiation and its potential to disrupt meristematic tissues at the genetic and/or epigenetic level. Moreover, the pronounced increase in proline content highlights the activation of osmotic-stress signaling pathways and stress-adaptive mechanisms in irradiated individuals. Such physiological adjustment may constitute a secondary benefit by facilitating the identification of genetic variants with enhanced tolerance to abiotic stresses at higher radiation doses. Moreover, the current study enhances the understanding of mutation breeding in fenugreek (*Trigonella foenum-graecum* L.) by identifying an effective range of γ-irradiation doses (50–500 Gy) that induces functional, morphological, and physiological variation while minimizing phytotoxic effects. Although γ-irradiation has been used on various crop species, precisely determining an optimal mutagenic threshold for fenugreek provides a framework for balancing genetic variability with plant survival. Notably, the observed dose-dependent increase in proline accumulation indicates a previously underexplored physiological response, suggesting that irradiation not only promotes novel variation but also activates pathways for adaptation to osmotic challenges.

In this context, γ-ray–mediated mutation breeding emerges as an innovative and cost-effective strategy for augmenting genetic diversity in countries endowed with rich medicinal and aromatic plant resources, such as Türkiye. To fully exploit the agronomic potential and stability of the induced variants, subsequent work should integrate advanced breeding methodologies across the M2 and M3 generations. Specifically, molecular marker-assisted selection (MAS) may facilitate the rapid identification and accumulation

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of desirable alleles. At the same time, transcriptome profiling under both stress and non-stress conditions can elucidate the regulatory networks underlying trait variation. Simultaneously, high-throughput phenotyping in field trials utilizing remote sensing, hyperspectral imaging, and automated data collection will assess agronomic performance, yield stability, and phytochemical profiles under actual cultivation conditions. This multidisciplinary approach, which integrates controlled mutagenesis with genomic, transcriptomic, and phenomic tools, holds great promise for accelerating the development of elite fenugreek lines with improved bioactive compound content, enhanced stress resilience, and increased market value.

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