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Determination of Effective Mutation Dose (ED50) to be Used in Variety Treatment in Tea Plant

Burcu GÖKSU KARAOĞLU[1*], Yıldız AKA KAÇAR[2], Yaprak TANER KANTOĞLU[3], Burak KUNTER[3]
Keziban YAZICI[1]

[1] Recep Tayyip Erdogan Universtiy, Faculty of Agriculture, Department of Horticulture, Türkiye
[2] Cukurova University, Faculty of Agriculture, Department of Horticulture, Türkiye
[3] Turkish Energy, Nuclear and Mineral Research Agency, Türkiye

Sorumlu Yazar/Corresponding Author
E-mail: burcu.goksu@erdogan.edu.tr

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Abstract

Although tea is cultivated only in the Eastern Black Sea region of Türkiye, all tea plantations in the area have been established from seeds since the beginning of tea farming. This has negatively affected both yield and quality. The high level of heterozygosity in tea plants and the presence of self-incompatibility mechanisms prolong breeding efforts. Mutation breeding is used as a fast and practical method to induce genetic variation. In leading tea-producing countries such as China, India, Kenya, and Sri Lanka, commercially important tea cultivars have been developed through mutation breeding. However, no such studies have been conducted in Türkiye. This study aimed to determine the effective mutation dose that can be used to induce variation in tea plants. The research was conducted between 2020 and 2021 at the Tea Research Greenhouse of the Faculty of Agriculture, Recep Tayyip Erdoğan University. The plant material used was 3–4-node shoots of the Zihni Derin tea cultivar. These shoots were irradiated with 0, 10, 20, 30, 40, 50, 60, and 70 Gray (Gy) doses using the “Ob-Servo Sanguis Co-60 Research Irradiator” gamma irradiation device at the Turkish Energy, Nuclear and Mineral Research Agency. Single-node cuttings obtained from the irradiated shoots were planted in a peat-vermiculite mixture. Survival and rooting rates of the tea cuttings exposed to different gamma doses were recorded, and shoot length (mm) was measured in the growing tea seedlings. Survival and rooting rates decreased with increasing radiation dose. Based on a probit regression analysis of shoot length, the “Effective Mutation Dose” for tea was determined to be 11.45 Gy. This study represents the first mutation breeding research on tea in Türkiye and is expected to provide a foundation for future work in this field.

Keywords: *Camellia sinensis* L., cultivar development, efficacious radiation rate, gamma ray, mutation breeding

Çay Bitkisinin Çeşit Islahında Kullanılacak Etkili Mutasyon Dozunun (ED50) Belirlenmesi

Öz

Çay bitkisi Türkiye’de sadece Doğu Karadeniz bölgesinde yetiştirilmekle birlikte yetiştiriciliğinin bu bölgede yaygınlaşmaya başladığı tarihten itibaren bahçeler tohumla tesis edilmiştir. Bu durum verim ve kaliteyi olumsuz yönde etkilemektedir. Çay bitkisinde heterezigoti oranının yüksek olması ve kendine uyuşmazlık mekanizmasının varlığı ıslah çalışmalarının süresini uzatmaktadır. Hızlı ve pratik bir şekilde genetik çeşitlilik ortaya çıkarmak amacı ile mutasyon ıslahı yöntemi kullanılmaktadır. Çay üretim sıralamasında ilk sıralarda yer alan Çin, Hindistan, Kenya ve Sri Lanka gibi ülkelerde mutasyon ıslahı ile ticarete konu olan önemli çay çeşitleri geliştirilmiştir. Ülkemizde ise bu konuda bir çalışma yapılmamıştır. Bu çalışmada, çay bitkisinde varyasyon oluşturmada kullanılabilecek etkili mutasyon dozununun belirlenmesi amaçlanmıştır. Çalışmamız Recep Tayyip Erdoğan Üniversitesi, Ziraat Fakültesi, Çay Araştırma Serasında 2020-2021 yıllarında yürütülmüştür. Materyal olarak kullanılan Zihni Derin çay çeşidine ait 3-4 gözlü sürgünlere Türkiye Enerji Nükleer ve Maden Araştırma Kurumu’nda İzotop marka “Ob-Servo Sanguis Co-60 Research Irradiator” model gama ışınlama cihazı ile 0, 10, 20, 30, 40, 50, 60 ve 70 Gray (Gy) dozlarında ışınlama yapılarak tek gözlü olarak alınan çay çelikleri torf-vermikulit karışımına dikilmiştir. Farklı dozlarda gama ışını uygulanan çay çeliklerinin canlılık ve köklenme oranları belirlenmiş büyüyen çay fidanlarında ise sürgün uzunluğu (mm) ölçümleri yapılmıştır. Çay çeliklerinin canlılık ve köklenme oranları doz artışına bağlı olarak azalma göstermiştir. Sürgün uzunluğuna göre yapılan probit regresyon analizi sonucunda ise çayda ‘’Etkili Mutasyon Dozunun’’ 11, 45 Gy olduğu tespit edilmiştir. Bu çalışma Türkiye’de çayda mutasyon ıslahı konusunda yapılan ilk çalışma olup bundan sonraki çalışmalara ışık tutacak niteliğe sahiptir.

Anahtar Kelimeler: *Camellia sinensis* L., çeşit geliştirme, etkili radyasyon oranı, gama ışını, mutasyon ıslahı

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1. Introduction

Tea is obtained by processing fresh leaves and is the most consumed beverage in the world after water. In the world, 28,191,556.18 tons of tea are produced from of 5,245,319 hectares. In our country, 1,450,000 tons of tea are produced from an area of 82,247 hectares. In terms of tea production, Türkiye ranks 4th after China, India and Kenya (FAO, 2021). Tea production in Türkiye is carried out only in the Eastern Black Sea Region, and Rize province, which has approximately 70% of the production. Rize has the highest production at 895,240 tons and followed by Trabzon, Artvin, Giresun, and Ordu provinces, respectively (TSI, 2022).

The tea plant is a cross-pollinated plant with a very high heterozygosity rate. It is generally self-incompatible and reaches its main yield after 4-5 years. For these reasons, obtaining the desired hybrids from selected parents in breeding programs becomes difficult. Moreover, in studies conducted with traditional breeding methods, the improvement of traits controlled by multiple genes is extremely difficult and time-consuming (Gunasekare, 2007; Ma et al., 2014; Singh et al., 2022). Mutation breeding is one of the practical and effective breeding approaches for improving the genetic structure of the tea plant (Sheasen, 2019; Sukirtharuban et al., 2020).

The primary goal of mutation breeding is to develop superior plant varieties by modifying one or two major traits that enhance yield or quality, without compromising the agronomic performance or quality characteristics of well-adapted cultivars (Udage, 2021; Ahloowalia et al., 2004). The countries that have developed the highest number of mutant varieties using mutation breeding are China (817 mutants), Japan (500 mutants), India (345 mutants), Russia (216 mutants), and the Netherlands (176 mutants) (IAEA, 2023). This breeding method has been successfully applied in numerous species, including cherry (Kunter, 2013; Özyiğit et al., 2021), mandarin (Çimen et al., 2019; Turgutoğlu, 2019), pummelo (Rana et al., 2020), fig (Ayar, 2022; Özen et al., 2015), tangerine (Turgutoğlu and Baktır, 2019), grapevine (Marasalı et al., 2003), papaya (Chan, 2009), Japanese pear (Sanada, 1988), mangosteen (Rostini et al., 2003), and tea (Sukirtharuban et al., 2020).

In tea plants, spontaneous (natural) mutations can occur (Tan et al., 2015), and variation can also be induced through the application of physical and chemical mutagens, such as gamma rays (Singh et al., 2022). Gamma radiation is considered a highly effective method for inducing biochemical variations in tea (Kamau et al., 2014; Singh et al., 2022). The high frequency of mutants obtained from gamma-irradiated plants demonstrates that gamma rays are highly successful and effective method for developing new cultivars. Desirable lines and varieties have been developed using this technique (Nakagawa, 2009; Sukirtharuban et al., 2020).

The genetic diversity induced by mutagens in tea plants supports the potential of this technique in tea breeding, as noted by Khan (2012), while Banerjee (1992) emphasized the need for wider use of this method to enhance variation. Further studies using tea plants are needed to better understand the altered gene characteristics and to evaluate the effects of irradiated populations on tea yield and quality (Singh et al., 2022).

The primary goal of determining an effective mutation dose is to create rich phenotypic variation and develop new genotypes with superior traits. However, increasing mutagen doses also raises the frequency of harmful mutations and physiological damage. Therefore, it is essential to determine the dose that reduces plant growth by 50% known as the median growth reduction dose before starting breeding studies. The optimal dose varies by species and cultivar (Sarıçam et al., 2018; Yılmaz & Soysal, 2021). In vegetatively propagated plants, the most effective dose range should fall between those that are non-lethal in more than 50% of individuals and those that do not reduce growth by more than 50% (Nwachukwu et al., 2009).

In Sri Lanka, the lethal dose (LD50) value for tea micro-cuttings was determined using γ-rays (Co60) (Sarathchandra & Pieris, 2001). In China, Chen et al. (2007) reported an increase in improved clones among 45 clones developed using controlled hybridization and mutation methods. Sheasen (2019) noted that regionally and nationally registered clones were developed through Co60-based irradiation of tea seedlings and seeds. In Kenya, Kamunya et al. (2012) reported the inclusion of mutagens in the Tea Breeding Program to develop new genotypes tolerant to stress factors.

Additionally, mutation breeding studies in various countries have led to the development of early-maturing tea cultivars with high liquor quality, disease resistance, and suitability for green tea production (Yang et al., 2003; Kudo and Futsuhara, 1974; Nakayama, 1973). According to data from the International Atomic Energy Agency, the high-yielding and high-quality tea variety 'Fufeng' was registered in China in 1997 following gamma irradiation, while in Japan, the self-compatible variety 'Tea Noh Pl 2' was registered in 1998 (MVD, 2024).

In Türkiye, some of the issues in tea production include the establishment of tea gardens from seeds, low yield and quality, and the lack of tea varieties that can be recommended to farmers. In countries with which Türkiye compete in tea production, many breeding studies have been conducted, and many tea varieties obtained from these studies have become commercial products. In our country, mutation breeding has been carried out to develop varieties in many fruit species, particularly citrus. However, no such study has been conducted for tea. This study aims to determine the effective mutation dose for the Zihni Derin tea variety by using mutation breeding for the first time in tea breeding in Türkiye.

2. Materials and Methods

2.1 Material

As plant material, leaf cuttings measuring 5-6 cm in length with a single bud from the Zihni Derin (Fener-3) (*Camellia sinensis* L.) variety, registered by ÇAYKUR and selected from the Rize province, were used. The leaves of this variety are dark green in color with prominent veins between them. The leaves stand erect. The shoots are fleshy, crisp, and heavy in structure. The tendency to bend is minimal. The shoot formation is good and maintains its freshness for a long time. It has high adaptability. The underside of the bud and the first leaf is covered with fine hairs. It has a high branching ability. It forms a dense bush structure starting from the root region. The flower buds are large and showy, with a low seed-setting rate; it generally does not produce seeds. It is suitable for the production of oolong and black tea. The shoot formation period is moderately early.

2.2 Methods

In order to obtain the shoots for cuttings, the Zihni Derin (Fener-3) variety was pruned for rejuvenation in November 2019. By July 2020, the shoots had reached a semi-flexible form suitable for taking cuttings. These shoots were prepared in bundles, each 20-25 cm long. For each applied dose, 100 cuttings were used.

The tea shoots were irradiated with gamma rays at doses of 0, 10, 20, 30, 40, 50, 60, and 70 Gy using an "Isotope" brand "Ob-Servo Sanguis Co-60 Research Irradiator" gamma irradiation device at the Turkish Energy, Nuclear and Mineral Research Agency, Nuclear Energy Research Institute. After irradiation, the tea shoots were taken to the Tea Research and Application Greenhouse at Recep Tayyip Erdoğan University, Faculty of Agriculture, where they were planted in a peat-vermiculite mixture as 5-6 cm long single-bud leaf cuttings. Regular irrigation was carried out using a misting system. No rooting hormone was applied during the rooting stage of the cuttings.

After shoot formation occurred in the irradiated tea cuttings, the shoot length for each dose was measured using a caliper (OEM-KMP150). The data obtained from the study were analyzed using probit regression analysis to determine the 'Effective Mutation Dose' (EMD50). Additionally, to assess the effects of different radiation doses on the plants, the survival and rooting rates of the cuttings were calculated. The survival rate was determined by multiplying the ratio of the number of viable (root and shoot-forming) cuttings to the total number of cuttings by 100. Rooting percentage was calculated by dividing the number of rooted cuttings by the total number of cuttings and multiplying the result by 100.

3. Results and Discussion

3.1 Vitality and Rooting Rates in Cuttings

When examining the rooting rates of cuttings exposed to different doses of gamma radiation, it is observed that the rooting rate of the control group is 93.67%, the rooting rate at 10 Gy is 73.41%, the rooting rate at 20 Gy is 65.49%, and the rooting rate at 30 Gy is 13.60%. In tea cuttings exposed to gamma radiation at doses of 40, 50, 60, and 70 Gy, no rooting occurred (Table 1). In the tea cuttings exposed to a 30 Gy dose, a rooting rate of 13.60% was achieved; however, the buds in the leaf axils of the rooted cuttings lost their viability. It has been reported by many researchers that high doses of gamma radiation inhibit rooting and reduce root length (Karmarkar et al., 2001; Wi et al., 2007; Marcu et al., 2013; Sadhukhan et al., 2015).

Table 1. Viability and rooting rates of irradiated cuttings according to doses

Doses (Gy)	Viability Rate (%)	Rooting Rates (%)
0 Gy	92.40	93.67
10 Gy	46.24	73.41
20 Gy	7.60	65.49
30 Gy	0	13.60
40 Gy	0	0
50 Gy	0	0
60 Gy	0	0
70 Gy	0	0

Gy: Gray

In addition to the slowdown in growth and development of plants exposed to gamma radiation, increased doses have also resulted in plant mortality. In this study, cuttings that were able to form shoots and roots were considered viable. The group with the highest viability rate was the control group, with a rate of 92.40%. The viability rate for tea cuttings exposed to 10 Gy of gamma radiation was 46.24%, and for those exposed to 20 Gy, it was 7.60%, while the viability rate was 0% for tea cuttings exposed to doses of 30, 40, 50, 60, and 70 Gy (Table 1, Figure 1). Singh (1980) reported that tea clones of Chinese and Assam origin are generally more tolerant to gamma radiation compared to Cambodian clones, but tea clones lose their viability at doses above 2 krad. Dong et al. (1985) indicated that the viability and growth rates of tea cuttings decrease at doses above 1 krad, with the lethal dose being 2 krad. Many researchers have demonstrated that as the radiation dose applied in mutation breeding increases, the viability rate decreases (Sadhukhan et al., 2015; Alyanak, 2019; Muhammad et al., 2021; Hasim et al., 2021; Yarar et al., 2022).

Figure 1. Viability status of tea cuttings treated with different doses of gamma rays and the control group

3.2 Shoot Lengths and Regression Analysis

The shoot lengths of plants exposed to different doses of gamma radiation were measured with calipers 48 weeks later, in June 2021. The average measurements of shoot lengths are provided in Table 2. The shoot length was measured at 85.07 mm in the control group, 88.09 mm at a dose of 10 Gy, and 80.42 mm at a dose of 20 Gy. At radiation doses of 30 Gy and above, the buds in the leaf axils of tea cuttings lost their viability, and no shoot formation was observed in the plants. In this study, it was determined that the shoot length of plants treated with a dose of 10 Gy was greater than that of the control group and plants subjected to other dose treatments. This is an expected outcome within the concept known as low-dose stimulation.

The % probit values of the measured shoot lengths were determined from the Finney table. When examining the relationship between shoot formation and shoot length in tea cuttings exposed to different doses of gamma radiation, the effective mutation dose (EMD50) for shoot length was calculated to be 11.45 Gy according to probit regression analysis. The R^2 value was determined to be 72% within the confidence limits (Figure 2).

Table 2. Average shoot length and probit values of tea samplings treated with different doses of gamma irradiation

Doses (Gy)	Shoot Length Avg. (mm)	Probit Value (%)
0	85.07	6.04
10	88.09	6.18
20	80.42	5.99
30	0	0
40	0	0
50	0	0
60	0	0
70	0	0

Gy: Gray

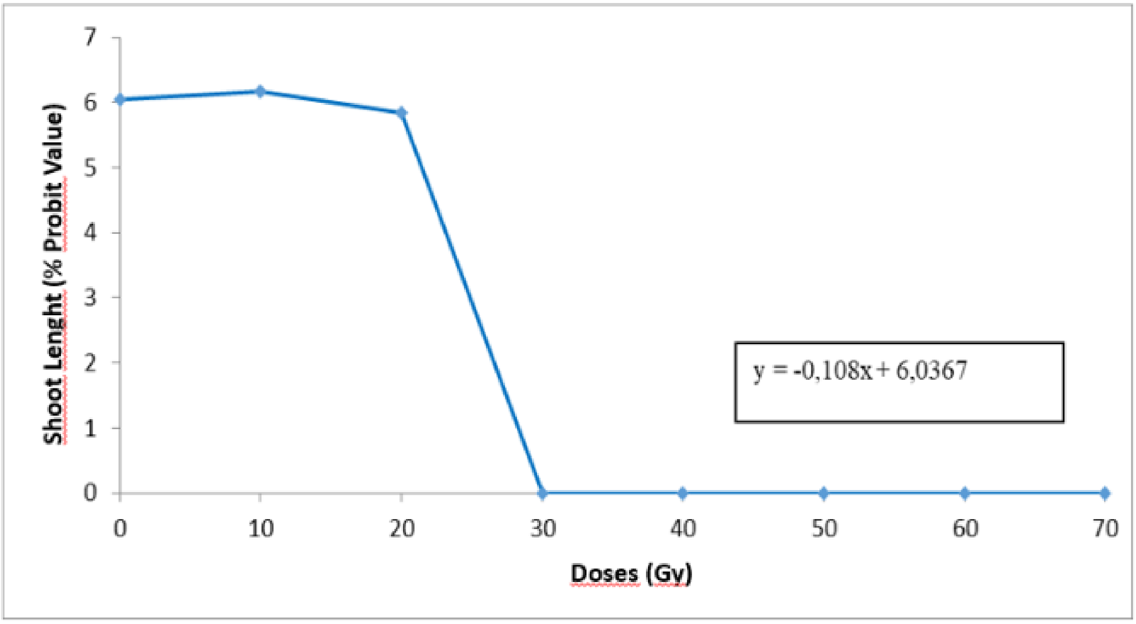

Figure 2. Shoot lengths and probit values of tea saplings according to doses

Many researchers have reported that increasing dose rates lead to a reduction in shoot length and plant height (Nakayama, 1976; Ali et al., 2005; Singh et al., 2022). Nakayama (1976) indicated that an increase in the applied dose of chronic gamma irradiation in black and green tea varieties resulted in a decrease in plant height. Ali et al. (2005) reported that at the end of the 52nd week, the plant heights of shoot cuttings exposed to gamma radiation at doses of 0, 10, 20, 30, 40, 50, and 60 Gy were 76.21, 74.60, 72.33, 71.12, 70.80, 68.61, and 65.70 cm, respectively. Singh et al. (2022) noted that an increase in gamma radiation doses applied to tea seeds resulted in a reduction in plant height, with doses above 2 Gy causing a disruption in normal cell division and leading to decreased plant height. However, in this study, it was found that the shoot length of plants treated with a dose of 10 Gy was greater than that of the control group and plants subjected to other dose treatments. This effect is expected and is due to the stimulatory effect of low-dose gamma radiation (Marcu et al., 2013; Yazıcı et al., 2016).

Babu (2008) reported that different doses of gamma radiation applied to single-node cuttings from seven different tea clones and three different biclonal seeds resulted in high variation at 3 krad in the BSS-2 genotype, and the highest dose tolerance in ATK-1 and UPASI-9 clones at 4 krad (40 Gy) in cuttings. Additionally, differences in chlorophyll content, leaf length, plant height, and branching patterns were observed depending on the mutagenic effect applied. In this study, it was found that plants lost viability at doses of 30 Gy and above.

In the study conducted by Mikautadze (1986), it was reported that low doses (10–15 Gy) of gamma radiation applied to tea seeds caused morphological changes (7.1–69.1%) mainly in leaves, and polyploid plants were formed at doses above 30 Gy, particularly around sublethal and lethal levels. Dong et al. (1985) determined that the germination rate decreased above the optimum dose of 5 krad for seeds and that the lethal dose was above 7 krad. Except for the Fudingdabai variety, the survival rate and growth rate of cuttings decreased at doses above the optimum (0.5–1 krad), and the lethal dose was found to be 2 krad. It has been reported that low-dose (<4 krad) irradiation positively affects pollen germination, and the LD50 dose was determined to be around 12 krad. Large-leaved varieties are more sensitive to radiation than small-leaved varieties. Rashid and Alam (1976) reported that the maximum dose for cuttings was 800 rad and for seeds was 3,000 rad in their study on the effects of low and high-dose gamma radiation on tea cuttings and seeds. Furthermore, it was observed that 400 rad of radiation applied to seeds had a positive effect on plant growth. The results obtained in this study to determine the effective mutation dose for the Zihni Derin tea variety are consistent with that of previous research.

4. Results

This study, the first of its kind in our country to apply mutation breeding techniques to tea, has determined the "effective mutation dose" for the Zihni Derin variety. This research is also significant as it serves as a starting point for researchers who will conduct studies on tea. Based on the information obtained, it has been determined that gamma radiation doses of 11–12 Gy can be effectively used in mutation breeding studies aimed at creating variation in tea, depending on the power and efficiency of the radiation source and the plant genome.

Author contribution

Göksu Karaoğlu, B: Follow-up of the study, finalization and writing the article; Aka Kaçar, Y: Supervision of the execution and progress of the study, writing control; Taner Kantoğlu, Y: Design of the research method, oversight of the writing; Kunter, B: Analysis of the data, oversight of the writing; Yazıcı, K: Planning the study, oversight of the work and writing

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Conflict of Interest Declaration

The authors declare that they have no conflict of interest.

Ethical standards

No Ethics Committee Approval is required for this study.

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