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Fraser Photinia shoot explantation *in vitro*: Effects of two distinct gamma-ray sources and identification of the optimal mutation dose

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ABSTRACT

Because of its beautiful qualities and ability to withstand harsh conditions, Fraser photinia (*Photinia* × *fraseri* cv. Red Robin) is frequently used as an ornamental plant in garden designs. The efforts to create new, highly marketable variations of the species have begun to increase in response to the growing ability of the current kinds to adapt to changing climatic circumstances. For this species, which is susceptible to *in vitro* propagation, the *in vitro* mutation breeding technique holds significant promise for increasing the current variety. It is essential to ascertain whether ionizing gamma ray sources are suitable for in vitro mutation investigations on Fraser photinia. To achieve this, *in vitro* shoot explants were exposed to a total of thirteen different radiation doses using ^{60}Co (dosage rate: 235 Gy/h) and ^{137}Cs (dosage rate: 821 Gy/h) gamma ray sources. The number of leaves and shoot length in *in vitro* plantlets were assessed thirty days after irradiation, and linear regression analysis was used to get the effective mutation dose (EMD_{50}) values. Based on the quantity of leaves, the EMD_{50} for the ^{137}Cs source ray application was 60.34 Gy, whereas the ^{60}Co source resulted in an EMD_{50} of 80.88 Gy. These findings demonstrated that the EMD_{50} difference was significantly impacted by the source power, irradiation duration, and the influence produced by the linear energy transfer value of the irradiation during tissue penetration.

1. Introduction

Plants are essential to landscape design for both functional and aesthetic reasons. The use of materials, particularly plant elements, has improved and diversified, and each section of the landscape design has been converted into areas that require specialization [1]. Because of the effects of climate change, the use of plant species that are not commonly found in the natural flora and are therefore not naturally grown in a region raises the value of landscaping [2, 3]. However, the plants chosen for landscaping should also be able to adapt to the local climate.

The evergreen, attractive woody shrub *Photinia* × *fraseri* Dress. (Fraser photinia, Fraser's photinia) is commonly planted in green spaces and can reach a height of three to five meters. This species, which is a member of the *Rosaceae* family, is a hybrid of Chinese *Photinia serrulate* and Japanese *Photinia glabra. Photinia* × *fraseri* is a popular hedge and decorative plant for parks, gardens, and roadside landscaping because of its striking leaf traits, colors, and quick growth. The leaves of this plant are either red (young leaves) or green (mature leaves). Because the young, bright red leaves contrast with the older, dark green ones, the perennial foliage is particularly stunning when it first sprouts [4]. Its perennial foliage is striking throughout the sprouting process because of its vivid crimson-red color, and its oblong, flat, glossy leaves are tolerant to cold. Large white terminal clusters are produced throughout the spring flowering season [5]. In addition to being aesthetically beautiful, this hedging plant gives the garden texture and acts as a useful windbreak. Additionally, Photinia plants' vibrant red leaves and open growth attract birds and other wildlife, fostering a happy coexistence of nature and the garden [6].

Photinia × *fraseri* 'Red Robin' is the most popular cultivar [7]. The hybrid plant originated from Fraser Nursery in Alabama (hence its name), but the 'Red Robin' was later developed in New Zealand [8]. A notable characteristic of photinia 'Red Robin' is its eye-catching leaves.

The evergreen shrub Photinia 'Red Robin', on the other hand, not only endures but flourishes in harsh environments. Because of its remarkable cold tolerance, Photinia 'Red Robin' is a great choice for areas with severe winters. The Red Robin cultivar, whose cold tolerance level is stated to be between -17.7 to -15.0 °C, can also be evaluated in different climatic conditions in urban planning as a plant with high temperature (+43.6 to +46.3 °C) [9]. Once

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established, it can endure dry spells without losing its aesthetic appeal. It can obtain moisture from deeper in the earth because of its extensive root system, which guarantees its survival during dry spells. Strong winds can make it difficult for plants to stay healthy and in shape. However, Photinia 'Red Robin' has exceptional wind resistance. It withstands wind effectively thanks to its strong branches and resilient leaves. As long as the soil drains properly, it can grow in a variety of soil types, including clayey, sandy, and loamy soils [10]. Improvement of clay soils with organic matter to allow roots to breathe and develop can be done by using organic matter such as biochar and barnyard manure or inorganic matter such as sand that lightens the soil structure. Although Red Robin photinia is quite tolerant to adverse environmental conditions, it is particularly sensitive to alkaline soils with high pH [7, 11] or high salinity conditions [12, 13].

While intensive research focuses on field crops and vegetables; breeding programs for ornamental plants, especially perennial plants, remain limited. The basis of plant breeding is the presence of genetic variation in the plant material under study. This variation can be obtained by selection, hybridization, and techniques from natural populations. According to the study by Özel et al. [14], “ortet selection” can boost Fraser photinia seedling performance many times. This study shows that genetic variation exists in the photinia species. The producers' profit rate will rise significantly if the fastest-growing ortets are chosen for the seedlings grown for landscaping since they will be able to reach a marketable size more quickly.

Studies mainly carried out in the last 50 years have shown that, especially in self-pollinating plants, genetic mutations confer significantly higher benefits in the development of traits than those governed by single genes and showing simple inheritance as reported by Anonymous [15], Waycott et al. [16], and Sağel et al. [17]. Abiotic stress tolerance, early, everlasting, and abundant flowering, altered photoperiodic response, and enhanced disease resistance are among the physiological properties that have been successfully mutated. Conventional breeding has contributed greatly to improving brush traits, but mutation breeding may provide an important complementary technique for improving stress tolerance and productivity. When combined with *in vitro* procedures, mutation induction and desired trait selection provide several benefits over traditional approaches [18].

Schum [19] reported that the use of gamma radiation in the use of physical mutagens is easy to use, highly permanent, accessible to target cells, and has an absence of toxic effects and damage. According to Maliga [20] and Ahloowalia [21], *in vitro* methods allow cells to be uniformly treated with physical and chemical mutagens and grown in homogeneous medium. *In vitro* cultures offer significant advantages for identifying somaclonal variation in plant breeding studies. *In vitro* mutagenesis experiments can be performed on large populations, in limited space, and at any time of the year. The chances of obtaining potent mutants are higher with *in vitro* mutagenesis. The main advantage of this method is that it avoids the formation of chimeras in M1V1. Large populations can be managed using tissue culture for mutagenesis treatment, selection, and cloning of specific mutants. It also enables quick subculture propagation cycles that separate mutated from non-mutated sectors [22]. Kane et al. [23], discussed the importance of mutation to produce phenotypic differences in Fraser photinia. They worked to develop an optimal propagation protocol in tissue culture to obtain a large number of plants for *in vitro* propagation of mutant plant material and selection.

When the studies on mutation breeding are evaluated in general, it is seen that ionizing radiation sources (such as gamma, X-ray, fast neutron, electron accelerator, and heavy ion sources) are used more heavily than chemical mutagens due to their ease of application [24]. Each ionizing radiation source is categorized by the Linear Energy Transfer (LET) value they have according to its ionization capacity [25]. Accordingly, ^{60}Co and ^{137}Cs, which are gamma ray sources, are sources with low LET levels [26, 27].

In the first phase of our studies to obtain new mutant lines tolerant to high salinity and pH levels from Red Robin photinia, it was planned to create new variations in *in vitro* shoot culture using the gamma irradiation technique. If we compare gamma radiation sources in terms of safety in the event of a nuclear or possible radiological accident, ^{60}Co sources have a lower risk of radiation pollution, contamination, and nuclear pollution compared to ^{137}Cs. When the two sources are compared in terms of energy, the energy, aggressiveness, and dose rate of ^{60}Co sources are higher than ^{137}Cs. Before starting the mutation breeding study with either gamma sources or other ionizing radiation sources, it is necessary to determine the EMD_{50} specific to the genotype to be studied as a preliminary study for the creation of the main mutant population [28, 29].

In the study whose results are presented here, it was aimed to compare the effects of the source effect as a result of irradiating the *in vitro* shoots of the Fraser’s photinia cv. Red Robin with two different gamma ray sources, ^{60}Co and ^{137}Cs, which have different dose rates and energies, and to determine the EMD_{50} according to both sources.

2. Materials and Methods

2.1. Material

In vitro Red Robin photinia shoots obtained from a commercial nursery company producing Fraser photinia via tissue culture were used as material in the research.

2.2. Methods

2.2.1. Plant culture conditions and multiplication of shoots

In this study, explants taken from stock cultures available in our laboratory were used. Therefore, no pre-sterilization process was performed on the explants. Main and lateral shoots were cut into nodal segments of similar size (7-8 mm). First proliferation media prepeared according to Larraburu et al. [30], the basic culture medium (BM) included 0.7% agar, 100 mgl^{-1} myoinositol, B5 vitamins [31], 3% sucrose, and Murashige and Skoog (MS) salts [32]. After bringing the pH down to 5.8, it was autoclaved for 20 minutes at 121°C. Explants transferred to 660 ml flasks with 50 ml of medium, supplemented with 2.2 µM BA and 0.5 µM IBA concentration. The cultures were incubated under LED illumination (55 µmol m−2 s−1) with a 16-hour photoperiod at 24 ± 2 °C and 55-60% relative humidity in a growth chamber. Shoot pieces of approximately 2 cm in length with a node on them were propagated in proliferation medium. Red Robin shoots that were sub-cultured 4 times, every four weeks, were transferred to MS [32] medium explained below.

2.2.2. Determination of Effective Mutation Dose

Two cm-long photinia shoots were cultured *in vitro* in the nutrient medium, and they were irradiated in eleven different doses (0, 10, 20, 30, 40, 50, 60, 70, 90, 110, and 130 Gy) with ^{60}Co (dose rate: 235 Gy/h) and ^{137}Cs (dose rate: 821 Gy/h) gamma ray sources within the Nuclear Energy Research Institute of the Turkish Energy Nuclear and Mineral Research Agency (TENMAK-NÜKEN) (Figure 1).

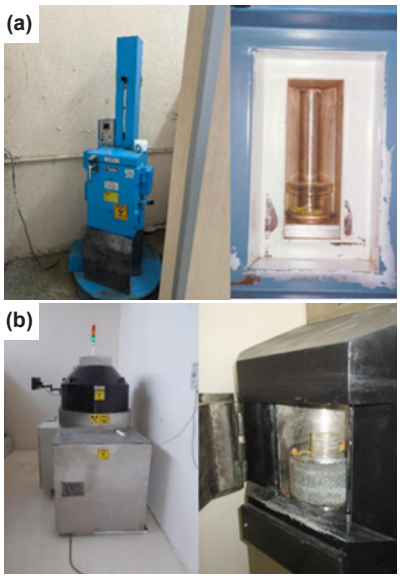

Figure 1. ^{137}Cs Research Irradiator (a); Ob-Servo Sanguis ^{60}Co Research Irradiator model (b).

As a general approach for *in vitro* explants, irradiation doses between 0 and 100 Gy are preferred [17]. However, in order to determine the dose response of the current genotype, which has not been studied before, irradiation was also performed with doses up to 130 Gy in 20 Gy increments after the 70 Gy dose. For each gamma ray and treatment dose, 30 *in vitro* shoots were used in three replication (each replication has 10 explants). The shoots were placed in sterilized nutrient hormone free MS media in autoclavable plastic bags to ensure that they fit easily into the irradiators (Fig. 2a). After irradiation, all explants were rapidly transferred to the fresh proliferation medium. This step aimed to protect the explants from the toxic effects of radicals expected to be formed during irradiation. The cultures were incubated in the controlled climatic room at 24 ± 2 °C (Fig. 2b). On the 30th day following irradiation, the shoot length and number of leaves were determined according to doses. Physical effects, including damage such as yellowing, darkening, and shoot death, caused by radiation application were also observed in explants during the period following irradiation.

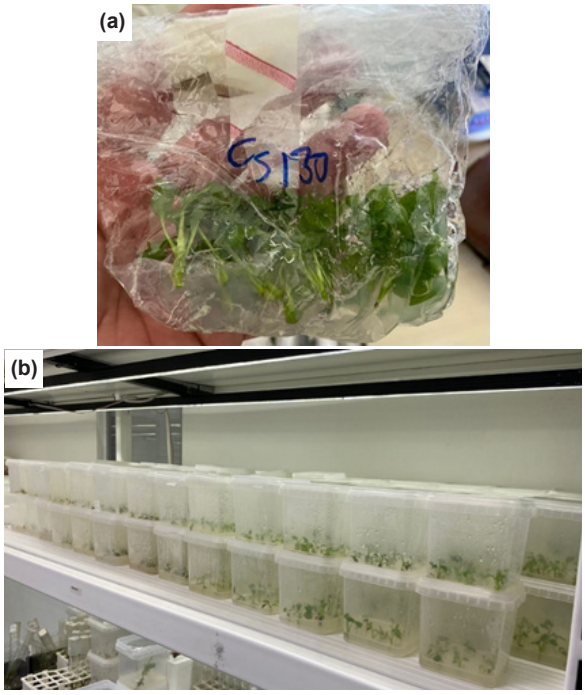

Figure 2. *In vitro* photinia shoots prepared for irradiation treatments (a); Incubation of photinia cultures after irradiation (b).

The shoot length and number of leaves data obtained after irradiation from both ionizing radiation sources were subjected to linear regression analysis to determine the EMD_{50} of the plant sample. EMD_{50} is calculated through the use of linear regression analysis by the Microsoft Excel software program [33]. In linear regression analysis, data were analyzed within a 0,95 confidence limit.

3. Results and Discussion

After irradiation at eleven different doses (control included) with ^{60}Co and ^{137}Cs gamma ray sources, all irradiated shoot tissues were sub-cultured to the fresh proliferation media to obtain shoot length evaluation and new leaves.

The study compared the shoot length and number of leaves of the test group, which received varying doses of gamma rays in both gamma ray sources, with non-irradiated non-irradiated shoots (control group). After irradiation at thirteen different doses with ^{60}Co and ^{137}Cs gamma ray sources, young leaves developed and shoot growth obtained from 30 shoots of each dose were counted 30 days after irradiation. Figure 3 shows the growth and development of irradiated photinia shoots. As a result of the observations, it was observed that the physical damage in the explants irradiated with the ^{137}Cs irradiation source was less than that of the ^{60}Co gamma source. In the ^{137}Cs source, a decreasing trend in leaf number was observed after 50 Gy application. According to Figure 4, which shows the results of the counting conducted on the 30th day following irradiation, there were notable decreases in the number of leaves as the irradiation dose increased.

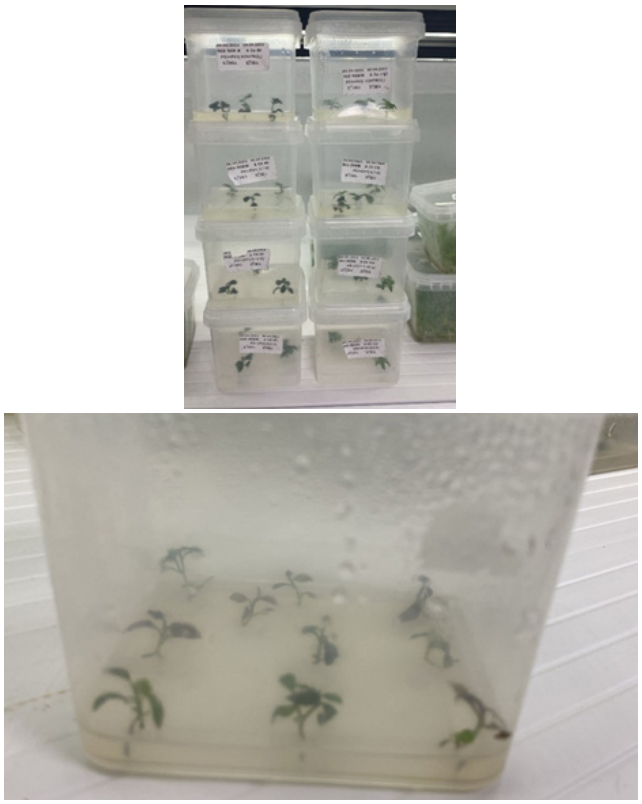

Figure 3. *in vitro* Fraser's photinia shoots developed under different irradiation treatments

This decreasing trend in shoot length was evident after a 60 Gy dose of the ^{137}Cs source. On the other hand, it was determined that the shoot length and leaf number values of the explants continued, albeit decreasingly, including the 130 Gy dose of the ^{60}Co source (Table 1). It was determined that there was a serious growth and development retardation with the 80 Gy application of the ^{60}Co source. However, it is observed in Figure 4 that the stable retardation in the cesium source could not be provided in the cobalt source. The regression analysis result performed with the data obtained as a result of irradiation with the gamma source is given in the formula (For 1.) below. In this formula, y represents

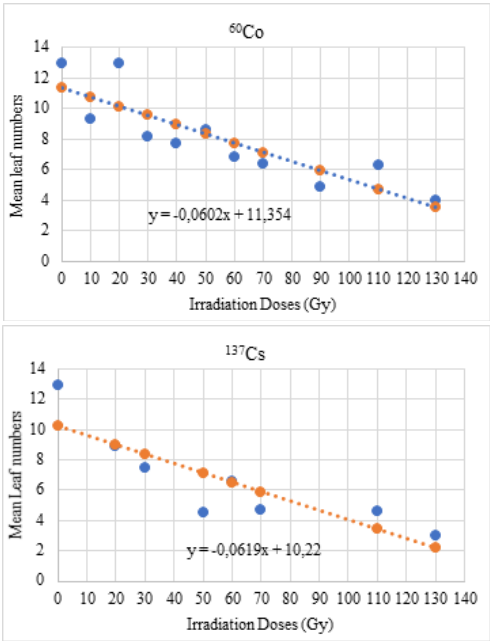

Figure 4. Graphs of leaf numbers of explants irradiated with two different gamma sources according to doses

the 50% value of the leaf number and shoot length obtained in the 0 Gy application, which is the control, while the value defined as x defines the EMD_{50}. Since the r^2 values of the average shoot values obtained after irradiation with both ionizing radiation sources were obtained below the acceptance limit of 0.74, the EMD_{50} calculation was determined based on the number of leaves that are important for this cultivar. Accordingly, the EMD_{50} was determined as 60.34 Gy as a result of ^{137}Cs (For 1). However, after being exposed to the ^{60}Co source, the EMD_{50} changed to 80.88 Gy (For 2.).

y = -0,0619x + 10,22 For 1.

y = -0,0602x + 11,354 For 2.

Today, studies on mutation breeding are carried out in many species around the world using different irradiation sources (low and high energy) [34]. Factors such as the type of biological material used

Table 1. Shoot length and leaf number of photinia explants irradiated with two different gamma sources according to doses

^{60}Co			^{137}Cs		
Dose (Gy)	**Mean shoot length (cm)**	**Mean leaf number**	**Dose (Gy)**	**Mean shoot length (cm)**	**Mean leaf number**
0	1.80	12.97	0	1.8	12.97
10	1.11	9.30	10	infected	Infected
20	1.57	13.00	20	1.24	8.87
30	1.66	8.13	30	1.29	7.43
40	1.54	7.70	40	1.01	3.43
50	1.41	8.57	50	1.56	4.53
60	1.17	6.87	60	1.48	6.57
70	1.16	6.43	70	1.32	4.67
90	1.02	4.90	90	1.05	8.60
110	1.07	6.30	110	1.21	4.63
130	1.07	4.00	130	1.05	3.00

(p value was 0,006 both cobalt and cesium sources, Sx cobalt: 1,54; Sx cesium: 1,77)

in the study (seed, *in vitro* explant, cutting, etc.) and, its water content, the LET value of the source to be irradiated, the physical and chemical effects created by the radiation source the half-life of the source used in irradiation and its dose rate and the oxygen level of the environment during application are important [26]. When the obtained findings are evaluated, the first parameter that comes to the fore is the dose rate of the source. Because the ^{137}Cs source has a higher dose rate (820 Gy/h). Although its penetration into the tissue during irradiation seems lower than the ^{60}Co source. An effective dose distribution in a short time has been provided in line with the EMD_{50} obtained for the explants. The exposed dose rate of the ^{60}Co source is 232 Gy/h, but the irradiation time is longer when compared to the ^{137}Cs source and therefore, especially for *in vitro* material the formation of free radicals in the nutrient medium is accelerated and the amount of oxygen decreases accordingly, and it is possible to create different effects on the explants [35, 36]. Similar effects and findings have been revealed in studies conducted by different researchers [26, 27, 36, 37].

Conclusions

In vitro mutation breeding studies continue to be widely used to increase the gene pool and shorten the breeding period. In this study, *in vitro* shoot explants of Red Robin photinia were used. In order to conduct mutation breeding studies with a mutant population with a wide variation, the EMD_{50} should be determined according to the genotype, explant type, and mutagen source to be used in mutation induction. As a result of the irradiation of *in vitro* shoot explants with ^{60}Co and ^{137}Cs gamma radiation, differences in absorbed doses were revealed due to source power, linear energy transfer values, and irradiation duration. Accordingly, the EMD_{50} dose limits that should be applied for the creation of an effective *in vitro* mutant population are;

- For the ^{60}Co source, the EMD_{50} is 80.88 Gy; the 10% lower and upper limits of this dose are determined as ~73 - 89 Gy.
- For the ^{137}Cs source, the EMD_{50} is 60.34 Gy; the 10% lower and upper limits of this dose are determined as ~54 - 66 Gy.

The number of leaves was found to be an appropriate parameter in determining the EMD_{50}, but the shoot length did not give the expected critical value depending on the explant type and genotype response. In this study, the EMD_{50} in two different gamma radiation sources differed. The difference between the dose rate of the sources and the irradiation time of the samples depending on this dose rate, was observed clearly in terms of leaf numbers and shoot lengths. Thus, the importance of determining the EMD_{50} using the appropriate evaluation parameter in *in vitro* mutation breeding studies and the need for dose planning accordingly were revealed. It is expected that low-energy ionized sources, such as electrically activated X-rays and electron accelerators, will replace gamma sources, especially in the next decade. It is important to start mutation breeding studies by knowing the effect mechanisms of these sources to obtain successful results.

Author Contributions Statement

Dr. Onur Sinan Türkmen: Project design, experimental work, manuscript writing.

Dr. Kadriye Yaprak Kantoğlu: Project design, irradiation, analysis of experimental work data, manuscript editing.

Prof. Dr. Şeküre Şebnem Ellialtıoğlu: Project design, manuscript editing.

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