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Study of the effect of irradiation (^{60}Co) on M_1 plants of common bean (*Phaseolus vulgaris* L.) cultivars and determined of proper doses for mutation breeding

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ABSTRACT

The objective of this study is to determine the morphological changes and the proper dose of gamma irradiation for the mutation breeding program of common bean (*Phaseolus vulgaris* L.). The effects of different doses (100, 150, 200, 250, 300, 350, 400, 450 and 500 Gy) of gamma irradiation (^{60}Co) on seedling growth parameters and survival rates of the plants were investigated. In order to determine the proper dose and morphological changes; shoot length, root length, shoot fresh weight, shoot dry weight, root fresh weight, root dry weight, stem diameter, leaf width, leaf length, *chlorophyll* index and number of survival plants of M_1 plants were evaluated. It has been determined that the doses of gamma and the varieties used are significant on the number of surviving plants and plant growth parameters. It was determined that low doses gamma irradiation stimulatively effected on shoot fresh weight, shoot dry weight, root dry weight and root fresh weight. A negative correlation were obtained between all plant growth traits and stem diameter, and the stem diameters are thickened due to the increase in gamma doses. The applications of gamma rays were effected on survival plant rate considerably than the percentage of germination. Applicable proper dose for Efsane was determined as 318.22 Gy while appropriate dose for F16 was determined as 303.17 Gy.
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1. Introduction

Common bean (*Phaseolus vulgaris* L.) is an important crop for source of food and sustainable agriculture in the world. Over the years, classical breeding studies have led to the development of agricultural features of common bean cultivars, while limiting the existing features and developments in bean gene pools. Breeding and selection studies have caused a serious contraction in the genetic diversity of plant species (Tanksley & McCouch, 1997). In recent years, breeding trials aimed at obtaining uniform plants accelerated this process and caused many crops to become more sensitive to diseases, pesticides and abiotic stress conditions (Plucknett, Smith, Williams, & Murthi, 1983). García, Pena-Valdivia, Aguirre & Muruaga (1997) have once again demonstrated that breeding processes play an important role on loss of variation in wild and cultured beans. The genetic bottle-neck that occurs as a result of the continuous use of existing populations leads plant breeders to modernized breeding technologies. Mutation breeding has been an alternative technique preferred by breeders as it allows the possibility to form characteristics that do not exist in the nature or lost throughout the evolution.

It is possible to create new variations in a short time with the use of the mutation breeding method. The most used mutation breeding agent is physical mutagens such as ultraviolet (UV) light which leads to breaks on DNA double strain and deletions, gamma rays and neutrons have high energy radiation applications (Koornneef, 2002). Physical mutagens are an alternative breeding method to classical breeding methods as well as genetically modified organisms because their applications are relatively safe and cost-effective (Jain, 2010). According to International Atomic Energy Agency (2016) data, there are at least 3233 mutant cultivars. In context of products, maximum mutant variations were

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obtained in grains with 48% and vegetable species grown with mutation breeding remained at 3% (Mba, 2013). The ionizing radiation method, which began to be used in the early 20th century, plays an important role in the development of superior plants in more than 50 years of plant breeding studies (Kharkwal, 2012). The studies show that gamma rays can be used not only for breeding but also for post harvesting. Maraei and Elsawy (2017) reported that gamma rays applied after harvesting in the strawberry cultivars have positive effects on the antioxidant and phenolic content of the fruits.

Although there are several techniques used for mutation breeding, gamma ray technique is one of the most used physical mutagens (Sağel, Peşkircioğlu, & Tutluer, 2003; Sağel, Tutluer, Peşkircioğlu, Kantoğlu, & Kunter, 2009). Physical mutagens such as gamma rays are less risky to health than chemical mutagens because they require no application to remove mutagen from the material (Khan, Khan, Khan, Iqbal, & Zafar, 2000), because they are non-toxic and do not require detoxification after implementation (Mba, 2013). The ease of application plays an important role on widely spreading of the technique. 90% of the obtained mutant cultivars were obtained with this method (64% with gamma-rays, 22% with X-rays) (Jain, 2005, 2010). Several new cultivars have developed in Coriander (Salve & More, 2014) tomatoes (Sikder et al., 2013), Anthurium (Puchooa, 2005) and mungbean (Sangsiri, Sorajjapinun, & Srinivesc, 2005) etc. by using gamma rays. Gamma rays have been used for developing biotic-abiotic stresses tolerance and plant characteristics in the plants grown from the seed (Jain, 2010).

The most important point in mutation studies is to determine the appropriate dose for the species. Regression in growth parameters in the seedling obtained from radiation-treated seeds is usually an indicator of what the plant was damaged genetically. The LD_{50} dose should be determined to detect the most appropriate mutation dose (Predieri, 2001). In order to determine the appropriate dose, the first generational seedlings (M_1) and untreated seedlings are compared in terms of plant growth parameters and survival percentages (Mba, 2013). This data is used to determine the dose of LD_{50}.

In this study, it was aimed to compare the morphological differences that different gamma doses irradiated M_1 seedling of the common bean and to determine the proper gamma dose for common beans which is an important vegetable species.

2. Materials and method

2.1. Plant materials and methods

In the study, F16 and Efsane common bean seeds (standard cultivars) were used. The seeds used in the experiment were counted and placed in transparent petri dishes as 100 seeds for each control group and radiated at 0, 100, 150, 200, 250, 300, 350, 400, 450 and 500 Gy under ^{60}Co source (2190 kGy irradiation power/h) in Akdeniz University Gamma Rays Department, at 9 different dose levels. Irradiated seeds were sowed in viols which were previously prepared and contain a 1: 1 rate mixture of peat and perlite. Seedlings were growth in greenhouses belonging to Department of Horticulture, Akdeniz University under controlled conditions. Their germination rates were determined from the 7th day after the sowing until the 14th day. However, since the application of gamma irradiation has no effect on the germination rate, germination rates of seeds have not been evaluated. The heights of the plants were measured after being root-harvested at the 30th day. To determine the effect of gamma rays at different levels on morphological changes and proper dose; shoot length, root length, shoot fresh weight, shoot dry weight, root fresh weight, root dry weight, stem diameter, leaf width, leaf length, chorophyll index and number of survival plants of M_1 plants were measured.

2.2. Statistical analysis

The experiment was designed according to completely randomized with three replications and data analysis was carried out using the analysis of variance and SAS statistica computer package ($p \leq .05$) (SAS, 1985). SPSS 23.0 program was used to determine correlations between morphological features. LD_{50} values are determined by using the ROOT package (Brun and Radmakers, 1997).

3. Results and discussions

The number of studies investigating the effects of gamma rays on the plant development of common bean is quite limited. For this reason, a large dose scale has been tried to establish a basis for future work.

When the effects of gamma doses on plant root and shoot growth were examined (Table 1), it was observed that there was a sudden decrease in root length and root dry weight when 200 Gy gamma irradiation was applied in the Efsane cultivar. In the F16 cultivar, the root length gradually decreased in parallel with the increase of irradiation doses. However, the root fresh weight and root dry weight of the F16 cultivar increased to 200 Gy, and after that level it started to fall. Melki and Marouani (2010) reported that, low doses of gamma irradiation promoted root development on hard wheat, but root development was inhibited with increasing doses. A similar situation appeared when the shoot development of cultivars was examined. The shoot length of both cultivars showed a steady decrease. The most dramatic reduction in shoot length was achieved with 250 Gy application in the Efsane cultivar, while in the F16 cultivar it was manifested in the first application (100 Gy). Second dramatic decrease in the shoot length of the F16 cultivar was seen in the 350 Gy irradiation. The shoot fresh and dry weights of the Efsane cultivar was increased up to 200 Gy, then decreased. In the shoot fresh and dry weights of the F16 cultivar, minor increases and decreases were observed up to 350 Gy. But, there was a continuous decrease of 350 Gy and higher doses.

The effects of gamma doses on stem diameter, leaf development and chlorophyll index were given in Table 2. When Table 2 was examined, it was seen that the gamma applications had an enhancing effect on the stem diameter of the both cultivars. In both cultivars, after 300 Gy application, there was a decrease in the stem diameter. Generally, the stem diameter of plants exposed to gamma was found higher than control plants. This result showed that there was a relationship between body thickening and dwarfing in plants which gamma rays were applied. Depending on the increase in application dose, the leaf width and length of the cultivars have also decreased. Kayan and Eser (2004) found that chlorophyll mutations were also increased in the M_3 generation in the small-grained beans line due to the increased dose of gamma irradiation. In this study, in leaf growth values and chlorophyll index in both cultivars were increased in the highest gamma ray doses, but very few surviving plants could obtained in this doses. This can be explained that although very small amount of surviving plant is obtained as a result of gamma application at these high doses, the frequency of mutation formation increases in that plants. In high-dose applications, DNA damage and mutation rates are higher, but most of the plants die or have sterility (Koornneef, 2002; Preuss & Britt, 2003).

Correlations between morphological characteristics of bean M_1 plants are given in Table 3. As seen from Table 3, there are positive correlations between the plant morphological characteristics at different levels ($p < .01$, $p < .05$). However, it was determined that

Table 1
Effects of different gamma doses on shoot and root development in M_1 plants.

Cultivar	Treatment (Gy)	RL (mm)	SL (mm)	RFW (g)	SFW (g)	RDW (g)	SDW (g)
Efsane	control	210.80a	378.70a	1.24a	4.71a	11.23b	55.58c
	100	189.50a	310.20b	1.21a	5.41a	11.51a	58.63b
	150	154.80b	261.20c	1.08ab	5.20a	10.21c	58.71b
	200	95.47c	231.98c	1.40a	5.60a	8.76d	65.05a
	250	90.22cd	127.70d	1.35a	3.12b	8.53e	32.65d
	300	78.52cd	80.32de	0.86bc	1.87c	5.51f	5.20e
	350	65.45d	74.35e	0.65c	1.46c	4.47g	4.04f
	400	0	0	0	0	0	0
	450	0	0	0	0	0	0
	500	0	0	0	0	0	0
	$LSD_{\%0.05}$	27.102	48.896	0.3448	1.0888	0.0611	0.2849
F16	control	190.91a	459.06a	1.55bc	4.09ab	16.54f	47.78f
	100	208.94a	318.70b	2.35b	4.00b	25.12b	58.50b
	150	159.90b	260.95bc	3.73a	4.92a	27.20a	60.04a
	200	154.28b	231.97c	1.85bc	4.38ab	21.13c	51.16e
	250	161.32b	227.86c	1.70bc	4.62ab	19.33d	57.23c
	300	149.54b	211.97c	1.58bc	4.57ab	18.34e	53.30d
	350	121.79c	145.89d	1.56bc	3.06cd	15.30g	42.10g
	400	118.23cd	82.05e	1.32bc	3.79cd	13.31h	36.44h
	450	92.64de	77.75e	1.21c	2.57de	3.81ı	28.42ı
	500	82.50e	68.39e	0.86c	1.87e	0.77j	12.81j
	$LSD_{\%0.05}$	26.897	59.091	1.0934	0.84	0.4352	0.7568

Values in the same column followed by a different small letter are significantly different ($P < .05$).
RL: root lenght, SL: shoot lenght, RFW: root fresh weight, SFW: shoot fresh weight, RDW: root dry weight, SDW: shoot dry weight.

Table 2
Effects of different gamma doses on stem diameter, leaf development and chlorophyll index of M_1 plants.

Cultivar	Treatment (Gy)	SD (cm)	LW (mm)	LL (mm)	CI
Efsane	control	1.67d	67.70a	70.80ab	214.30a
	100	2.18c	67.84a	69.80ab	206.00a
	150	2.07cd	67.10a	72.30a	202.00a
	200	3.51a	61.53ab	61.58bc	171.60b
	250	3.05ab	52.97b	56.27c	133.40c
	300	2.93b	25.48c	25.05d	148.90c
	350	2.29c	27.84c	31.85d	149.40c
	400	0	0	0	0
	450	0	0	0	0
	500	0	0	0	0
	$LSD_{\%0.05}$	0.48	9.9641	10.225	22.079
F16	control	2.36c	81.56a	53.25ab	250.70a
	100	2.18cd	74.61a	54.94ab	222.40b
	150	1.80d	77.77a	57.68ab	195.00cd
	200	1.72d	75.24a	62.46a	211.30bc
	250	3.51a	70.99a	58.59a	200.60bc
	300	2.99b	78.53a	57.49ab	191.70cd
	350	2.89b	54.93b	57.14ba	171.30de
	400	1.76d	50.24bc	42.25bc	185.90cde
	450	3.02ab	24.70d	19.51d	125.80f
	500	3.23ab	37.33cd	29.22cd	162.70e
	$LSD_{\%0.05}$	0.4919	13.313	16.131	27.288

Values in the same column followed by a different small letter are significantly different ($P < .05$).
SD: stem diameter, LW: leaf width, LL: leaf lenght, CI: chlorophyl index.

there was a negative correlation between all morphological features examined by the stem diameter in both cultivars, but these values were statistically insignificant. As shown in Table 3, in relation to shoot and root development in M_1 plants of the Efsane cultivar; statistically significant positive correlation between RL and SL ($r = .944$, $p < .01$), between RFW and SFW ($r = .758$, $p < .05$) and between SFW and RDW ($r = .888$, $p < .01$). There is also a statistically significant positive correlation between CI and LW ($r = .756$, $p < .05$) and between CI and LL ($r = .756$, $p < .05$).

In M_1 plants of the F16 variety; statistically significant positive correlation between RL and SL ($r = .905$, $p < .01$), between RFW and SFW ($r = .667$, $p < .05$) and between RDW and SDW ($r = .710$, $p < .05$). As similar to the results of the Efsane cultivar; there is a statistically significant positive correlation between CI and LW ($r = .875$, $p < .01$) and between CI and LL ($r = .726$, $p < .05$).

The most important step in mutation studies is to decide which mutagen is to be used. The other important step is to determine the appropriate dose for the selected mutagen. To determine the appropriate dose, the control plants and the plants of the M_1 generation are compared according to their growth and survival rates. To determine the appropriate mutation concentration, the dose of LD_{50} should be determined (Mba, 2013; Predieri, 2001).

As a result of the statistical analysis, whereas the LD_{50} value for the Efsane cultivar was determined as 318 Gy, 303 Gy was determined for the F16 cultivar (Fig. 1). Yankulov, Isasi, and Abreu Ferrer (1980) stated that 75–175 Gy application is sufficient for lethality and mutation rates in 2 Cuban bean genotypes. However, the LD_{50} value obtained in this study was found to be different from that indicated by them. This comparison reveals again that genotypes respond differently to the mutation dose and that dose-scaling studies must be performed for large-scale studies.

Gamma ray applications reveal differences in the morphological characteristics of plants. These differences may occur in the later stages of plant growth, such as changes in seed colour (Moh, 1971) as well as on plant growth. Borkar and More (2010) have also observed this variation in the flower colour. They obtained bean plants with different flower colours such as white, red, purple, yellow and blue with 30, 40, 50 and 60 krad gamma irradiation and reported that high dose mutagen administration was more effective than low dose applications in the appearance of flower colour mutation.

The study, which was one of the first gamma ray studies in Morocco, was published in 1975 by Aboul-Saod & Omran. They used up to 50 doses of gamma in their study, and all applications except 50 krad stimulated plant germination, but at 50 krad the plants had just died. In terms of plant growth, they obtained similar results at all doses up to 30 krad application but they stated that plant length decreased significantly in 30 krad application. Aboul-Saod and Omran (1975) showed that 5, 10, 15 krad applications gave similar results with stimulant or control but 30 and 50 krad applications negatively affected plant growth. In this study, the

Table 3
Correlation coefficients among morphological traits of M_1 plants.

Cultivar		RL	SL	RFW	SFW	RDW	SDW	SD	LW	LL
Efsane	SL	0.944**	1							
	RFW	0.410	0.581	1						
	SFW	0.696	0.852*	0.758*	1					
	RDW	0.894**	0.926**	0.739	0.888**	1				
	SDW	0.698	0.862*	0.796*	0.992**	0.899**	1			
	SD	−0.715	−0.532	0.271	−0.116	−0.391	−0.128	1		
	LW	0.74*	0.896**	0.783*	0.944**	0.964**	0.967**	−0.288	1	
	LL	0.795*	0.883**	0.740	0.916**	0.953**	0.945**	−0.346	0.995**	1
	CI	0.927**	0.928**	0.278	0.755*	0.802*	0.734	−0.684	0.756*	0.756*
F16	SL	0.905**	1							
	RFW	0.548	0.437	1						
	SFW	0.745*	0.610	0.667*	1					
	RDW	0.694*	0.553	0.751*	0.606	1				
	SDW	0.851**	0.678*	0.719*	0.930**	0.710*	1			
	SD	−0.363	−0.280	−0.519	−0.417	−0.587	−0.336	1		
	LW	0.873**	0.823**	0.577	0.870**	0.583	0.865**	−0.362	1	
	LL	0.730*	0.612	0.530	0.809**	0.632*	0.847**	−0.315	0.907**	1
	CI	0.880**	0.892**	0.338	0.651	0.583	0.642*	−0.420	0.875**	0.726*

RL: root lenght, SL: shoot lenght, RFW: root fresh weight, SFW: shoot fresh weight, RDW: root dry weight, SDW: shoot dry weight SD: stem diameter, LW: leaf width, LL: leaf lenght, CI: chlorophyl index.
**Correlation is significant at the 0.01 level.
*Correlation is significant at the 0.05 level.

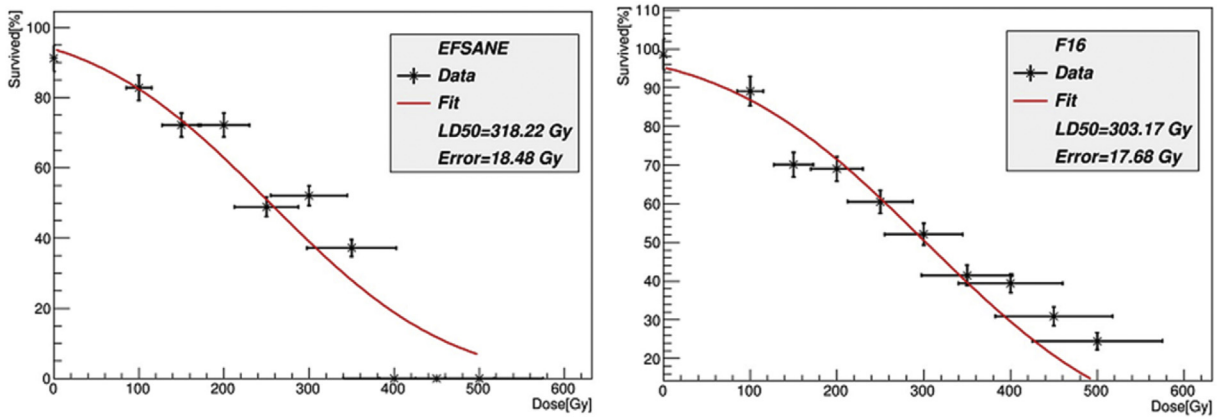

Fig. 1. The effects of gamma doses on the number of surviving plants and LD_{50} values.

growth of plants in the Efsane cultivar showed a decrease in parallel with the dose increase, and surviving plants in the applications of 400, 450, 500 Gy could not obtained. In the F16 cultivar, similar results were obtained in the plant development but the plants which survived the indicated doses could be obtained. Nevertheless, the development of plants exposed to 450 and 500 Gy doses was extremely poor. In this study, it was seen that application of high doses of gamma rays negatively influenced plant development, as in many studies previously done in different species (Girisha, 1993; Arunal, Prakash, & Sunil Kumar, 2010; Monica & Seetharaman, 2015; Velu, Mullainathan, & Arulbalachandran, 2012).

The germination percentages of seeds are used as parameters when the effectiveness of gamma rays is examined. However, as reported by Mejri, Saidi, Simier, Belhadj, and Mabrouk (2015), the results obtained in some studies (Melki & Marouani, 2010) show that gamma irradiation have no significant influence on germination percentages. Besides, It is stated that the reduction in the percentage of germination caused by the high doses of radiation applied may have resulted in a reduction in the amount of internal growth regulators depending on the radiation (Kiong, Ling Pick, Grace Lai, & Harun, 2008). In this study, gamma application in both cultivars did not have a significant effect on germination percentages but significant decreases in the number of surviving plants were detected with dose increase. As Bajaj (1970) put forward many years ago, gamma rays at low level (0.5krad) in the bean stimulate cell growth, but cell growth comes to a halt at the increase of irradiation dose. Bajaj (1970) had reported that gamma irradiation causes problems with RNA and protein synthesis, and then inhibits growth of plants *in vitro*.

4. Conclusion

In this study, it was aimed to determine the morphological changes occurring in common bean seeds exposed to gamma irradiation (^{60}Co) and the proper irradiation dose for mutation breeding studies. It has been seen that the doses applied in the study do not affect germination in both common bean cultivars but they cause significant differences in the number of surviving plants. The lowest dose, 100 Gy, stimulated the root development of the F16 cultivar. In future studies, it is thought that the determination of the effects of low gamma doses on common bean' root development will contribute to abiotic stress studies in particular. In this study, it was also determined that varieties had statistically

significant correlations between root and shoot development criteria and between leaf characteristics and chlorophyll index. Finally, it was determined that the radiation sensitivity of the cultivars are different by examining the surviving plants and plant growth parameters. This has once again proved that it is absolutely necessary to carry out a dose determination study before large-scale studies are carried out.

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