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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.

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

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