IMPROVEMENT OF BEAN PLANT TRAITS BY INDUCED MUTAGENESIS

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ABSTRACT

Phaseolus vulgaris is a leguminous plant with very high nutritional values and widely spread in Albania. Climatic changes have affected this plant to reduce its production and this experiment was designed to shorten the flowering time of Shijak variety bean plants, collected in Central Albania. We used as physical mutagens Cs-137 gamma radiation and we irradiated with 50 Gy, 100 Gy and 150 Gy. Also, we choose as chemical mutagens dES (Diethyl Sulphate), and we treated seeds with three concentrations: 0.0025M, 0.005M and 0.010M. The irradiated seeds were planted in the experimental field and the greenhouse during two consecutive years 2021 and 2022. The effect of physical and chemical mutagens on bean plants and pods was compared with untreated parental materials. Diverse traits were analyzed as germination ability, pigment change, chlorophyll content, pod characteristics, root system development etc. The best performance for pod length and width, seeds number and weight ware taken for the 50 Gy gamma radiation dose. This dose has increased production and the weight per unit. The chlorophyll content in M1 and M2 generation had evident differences according to irradiation doses. Photosynthetic pigments differ from one generation to another due to the action of physical mutagen. Since the chlorophyll content is an indicator of plant health, we observed that 100 Gy irradiation dose improves the condition of plants in the experimental field. For the first two dES concentrations the plants had a better development of the root system than the control.

Keywords: Induced mutagenesis, gamma radiation, dES, white bean

INTRODUCTION

Beans have exceptional energy values and contain almost all essential amino acids (Velu et al., 2012). Beans contain calcium, iron, phosphorus, magnesium, zinc and sodium, and it is an excellent source of protein, but beans are low in fat and contain no cholesterol (Coyne et al., 2003). Induced mutagenesis for a long time is considerate as a precious direction to create high quality and sustainable crop plants to environmental conditions (Sofkova et al., 2021). The experience of different research groups has shown that mutagenesis is an important for the creation of new plant variety especially at cereal, leguminous and many other crops. Also, the physical and economic possibilities of food are the important criteria of food security.

Induced mutations have played a major role in increasing food security, but for some bean varieties, radiation with doses from 300 Gy to 500 Gy are lethal (Singh et.al., 2005). In Albania the bean is important in the leguminous group. Climatic changes, especially in the recent years, have had a major impact on the production of this leguminous plant, affecting negatively it's production. For several years, the application of induced mutations has turned into the main way to create new cultivars with improved features by comparing them with the parents. Induced mutagenesis technology has been recently recognized as a valuable additional tool to create improved cultivars in agriculture (Horn et al., 2017). Mutant crop varieties are more adaptable to the environment, require less agricultural contributions, and are therefore more economical to grow and contribute to more environmentally friendly agriculture (Mba, 2013).

MATERIAL AND METHOD

The selected plant material was Shijak variety bean (*Phaseolus vulgaris*) seeds obtained from the National Seed and Seedling Entity which is the Genetic Bank of Albania. The experimental work was based to special protocols of the IAEA. The experimental work is organizing respectively in the Mutagenesis Laboratory, in an experimental field in the Fieri District (Albania) and in the greenhouse, part of the Department of Biotechnology, Faculty of Natural Sciences, University of Tirana. In the greenhouse experiment the seeds were planted in pots with soil with control of temperature and humidity. (Ylli et al., 2018; FAO / IAEA, 2018). For the treatment of the bean seeds was used the gamma radiation with Cs-137, which were applied in three different doses, 50 Gy, 100 Gy and 150 Gy (FAO / IAEA, 2018). The irradiated seeds and the control material were planted in the experimental field in randomized complete block (3m x 0,7m) and four replications, while in the greenhouse the seeds were planted in two replications.

We choose as chemical mutagens dES (Diethyl Sulphate), and we treated seeds with three concentrations: 0.0025M, 0.005M and 0.010M. The seeds were treated with this mutagenic solution for one hour, based in FAO / IAEA protocols (FAO / IAEA, 2018; Ylli, et al., 2013). CCM-200 type chlorophyll meter was used for the measurement and analysis of the chlorophyll pigment of the third level leaves of bean plants. Measurements of photosynthetic pigments were made in 25 plants for each dose.

RESULTS AND DISCUSSIONS

Measurements were done according to the above described protocol for all material taken from the experimental field in Fieri District and the greenhouse in Tirana, during two consecutive years 2021 and 2022. The analyzes of the bean plants materials *Phaseolus vulgaris* irradiated with three doses of gamma radiation 50 Gy, 100 Gy, 150 Gy as well as the control were done in the Induced Mutations Laboratory of the Biotechnology Department. The treated seeds were planted in the greenhouse and in experimental field (Figure 1) in the M1 generation and then in the M2 generation to see the changes in their development stages.

Figure 1. Bean seeds treated with gamma radiation planted in the greenhouse in two replications and in the experimental plot in four rows for each treatment dose

Different stages of plant development can be seen in Figure 2, which in doses of 50 Gy had set flowers 4 days earlier than the control, while in the dose of 150 Gy, flowers with a different color appeared compared to the control that has white flowers (Borkar et.al., 2010). During the vegetation period, have been recorded the phenological phase (germination, branching, flowering, beans, etc.).

Figure 2. Phases of phenological development of bean plants with different doses of radiation

Germination capacity was determined by observing the consecutive germination of each plants each week and by calculating the percentage of germination. By determining germination capacity, it's possible to determine the optimal dose that causes lower plant mortality, but at the same time causes highest desired percentage of mutagens. From the greenhouse material turns out that 100 Gy dose (83,3%) has the highest germination capacity in M1 generation, while 150 Gy dose (87,5%) has the highest germination capacity in M2 generation. Meanwhile in the experimental field 100 Gy dose (92,1%) has the highest germination capacity. These results show that the germination was affected by the plant material, genetic potential, seeds size, seeds germination ability etc. During this experimental work we have evaluated and measured chlorophyll pigment, and we observed different mutations under the influence of mutagens. We used CCM-200 chlorophyllmeter for measurements and analysis of photosynthetic pigments of bean leafs. Changes in the photosynthesis pigments content allow to evaluate their modification / reduction. Changes in the photosynthetic units respond to mutagenic as a protective mechanism in the most tolerant and resistant species. Since the chlorophyll amount was an indicator of plant health, we evaluated that 100 Gy irradiation dose improves the condition of plants in the greenhouse or in the experimental field. Figure 3 shows the large differences in the photosynthetic pigments measured in plants irradiated with the three doses used.

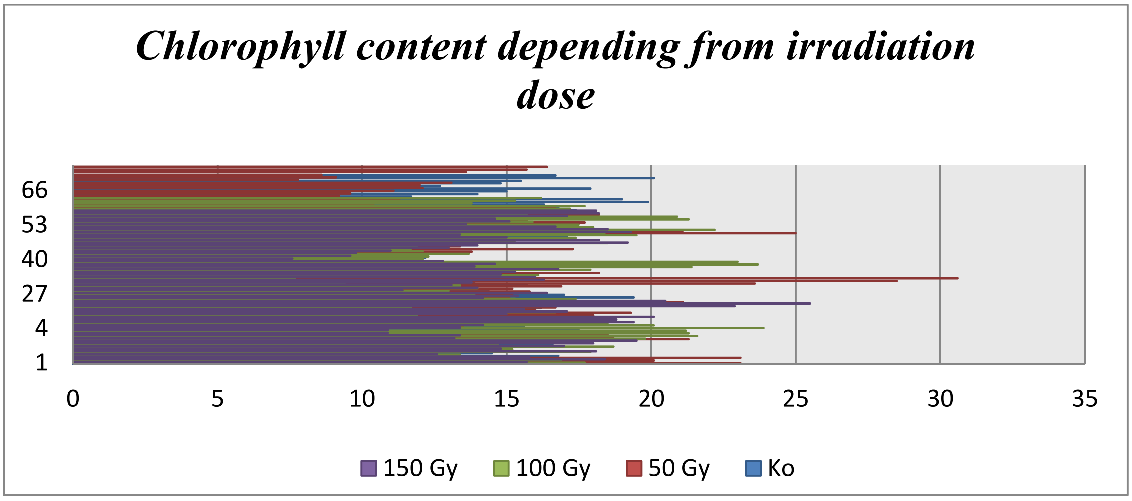

Figure 3. Chlorophyll content depending from irradiation dose

We also analyzed the root system of bean plants with different dozes and from the data obtained, stress caused by high temperatures develops a deeper root system that absorb more water, but the growth of the root system decreased the productivity.

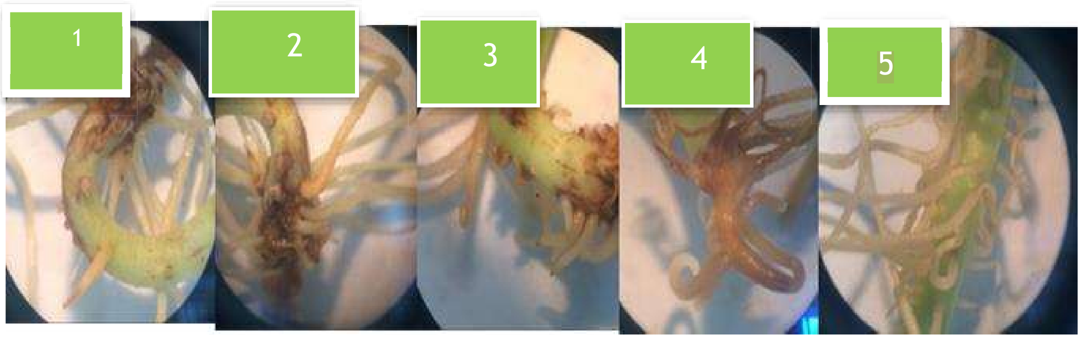

Figure 4. Analysis of the root system in plants treated with a dose of 50 Gy 15 days after planting

It's necessary to multiply roots hair to be able to use the same biomass more effectively or to stimulate a higher acid production in roots, to be able to main the same production or increasing it. Low phosphorus (P) availability and drought are the main constraints to common bean production.

The root system is an important factor for plant productivity in leguminous plants. During the experiment we followed the development of the roots after 12 days and after 15 days from germination measuring the length of the root. Stereomicroscopy with cameras was used to analyze the root system of plants and their development. As can be seen in the figure 4, 5 and 6, there are differences in their development depending on the doses of gamma radiation used.

From the measurements carried out in the laboratory concerning the plant germination capacity, we concluded that compared to the control, the seeds treated with the mutagen had longer roots. The seeds treated with the dose of 50 Gy were more developed than the control plants and had a greater length, so the treatment with the dose of 50 Gy affects the development of the plants. The 150 Gy dose reduces plant survival and is not effective in growing plants in Petri dishes.

Figure 5. Analysis of the root system in plants treated with a dose of 100 Gy 15 days after planting

Figure 6. Analysis of the root system in plants treated with a dose of 150 Gy 15 days after planting

From the measurements of leaf's, it was found that dES chemical mutagen with three different doses (0.0025M, 0.005M and 0.010M), affected the length of the leaves of the bean plants, but did not affect their width. The first dose of diethylsulfate (0.0025M), brought a higher increase compared to the control, where we get that with the increase of this dose, it brings a decrease from this average value (Kodhelaj *et al.,* 2021; FAO / IAEA, 2018). By processing the measurements performed on the leaves with the Anova program, we have analyzed the influence of the dES mutagen on the dimensions of the leaves of bean plants.

Table 1. Linear regression for leaf length after treatment with dES in M1 generation

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.206[a]	.043	.034	1.62251

a. Predictors: (Constant), dose of mutagen dES

In the linear regression analysis, it is determined that there is a stable relationship between the doses of the chemical mutagen and the length of the leaves because according to the Anova analysis, the significant value is less than 0.05 (Table 2). This relationship is determined by the correlation coefficient where only 4.3% of the length is determined by the dose of the chemical mutagen (Table 1). We can also determine this with the equation of a linear line where we have $y = 6.777+0.303x$. Thus, with the increase of one unit of the dose of the mutagen, the length of the leaf increases by 30.3% (Table 3).

Table 2. Analysis of variance for leaf length after dES treatment in M1 generation

Model		Sum of Squares	Df	Mean Square	F	Sig.
1	Regression	13.802	1	13.802	5.243	.024[b]
	Residual	310.638	118	2.633		
	Total	324.440	119			

a. Dependent Variable: the length of the leaf M1 with dES,
b. Predictors: (Constant), dose of mutagen dES

Table 3. Correlation coefficients for the association between dES and leaf length in M1

Model		Unstandardized Coefficients		Standardized Coefficients	T	Sig.
		B	Std. Error	Beta		
1	(Constant)	6.777	.248		27.343	.000
	dose of mutagen dES	.303	.132	.206	2.290	.024

a. Dependent Variable: leaf length M1/dES

Even in the linear regression analysis, it is determined that there is a stable relationship between the doses of the chemical mutagen and the length of the leaves, because according to the Anova analysis, the significant value is less than 0.05. This relationship is determined by the correlation coefficient where only 4.3% of the length is determined by the dose of the chemical mutagen. We can also determine this with the equation of a linear line where we have $y=6.777+0.303x$. We conclude that with the increase of one unit of the dose of the mutagen, the length of the leaf increases by 30.3%.

Table 4. Linear regression for leaf width after treatment with dES in M1 generation

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate
1	.082[a]	.007	-.002	.82072

a. Predictors: (Constant), dose of mutagen dES

Table 5. Analysis of variance for leaf width after dES treatment in M1

Model		Sum of Squares	Df	Mean Square	F	Sig.
1	Regression	.534	1	.534	.793	.375[b]
	Residual	79.482	118	.674		
	Total	80.016	119			

a. Dependent Variable: leaf length M1/dES
b. Predictors: (Constant), dose of mutagen dES

Table 6. Correlation coefficients for the association between dES and leaf width in M1

Model		Unstandardized Coefficients		Standardized Coefficients	T	Sig.
		B	Std. Error	Beta		
1	(Constant)	4.245	.125		33.863	.000
	Dose of mutagen dES	-.060	.067	-.082	-.890	.375

a. Dependent Variable: the width of the leaf M1/dES

As for the width, the correlation percentage is very low, where 0.7% of the width is determined by the dES dose (Table 4). This very weak relationship is also determined by the Anova analysis where the significant value is greater than 0.05 (Table 5).

If a linear line is constructed, it can be determined with the equation y = 4.245 - 0.060x, where with the increase of one unit of the dose, the values of the width of the leaves decrease by 6%. So in the four cases of materials obtained from treatment with chemical mutagens, the leaves had a difference in their length and in width they did not have any difference from the control, the leaves appear long compared to the leaves of plants pretreated with physical mutagens with the three doses (Table 6).

While for the width, the correlation percentage is very low where 0.7% of the width is determined by the dose of dES. This very weak relationship is also determined by the Anova analysis where the significant value is greater than 0.05. If we were to construct a linear line, we could define it with the equation y = 4.245 - 0.060x, where with an increase of one unit of the dose, the values of the width of the leaves decrease by 6%.

CONCLUSIONS

Induced mutagenesis on seeds of *Phaseolus Vulgaris* Shijak variety, treated with chemical mutagen dES with three concentrations as well as irradiated with gamma radiation of Cs-137 source with three doses has given positive influence on the change of traits and quality of the bean plant. The treatment with physical mutagens in the three doses of gamma radiation presents a higher average value for the body height and the development of the root system in the bean plants compared to the control, and this is also proven by the statistical analysis performed.

The use of induced mutagenesis for plant materials of physical mutagen treatments affected both values as leaf length and width, leading them to a circular shape. This was observed for the three doses used with the Cs-137 gamma radiation source.

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