PHOTOSYNTHETIC CHLOROPHYLL PIGMENTS IN *PHASEOLUS VULGARIS* TREATED WITH MUTAGENS

MALVINA KARÇINI (KODHELAJ), ARJANA YLLI AND NURIE PELIVANI

Department of Biotechnology, Faculty of Natural Sciences, University of Tirana, Albania

ABSTRACT

Beans (*Phaseolus vulgaris*) are considered one of major leguminous plants. In addition, they are characterised by a high genetic and significant variability, appropriate for agricultural production. Unfortunately, its production has significantly reduced due to climatic changes, and the abortion of flowers is concerning. The legumes do not survive at high temperatures and droughts period. E0fforts have been made to extend the flowering time and eliminate the abortion of flowers via induced mutation techniques applied on bean seeds. The Cs-137 gamma radiation, in three doses ranging from 50 to 150 Gy was applied to the seeds, in addition to the chemical mutagens dES diethylsulphate and EMS ethylmethansulphonate, in three concentrations, respectively. The IAEA protocols were followed when planting the irradiated and chemically treated seeds in the greenhouse and experimental field. The results obtained in the M1generation showed changes compared to the plants controlled for the physical and chemical treatments. During this time, chlorophyll pigments were analyzed and the results reported the mutagenic impact. The chlorophyll meter CCM-200 was used to measure the photosynthetic pigments in leaves. Regarding the chlorophyll content in plant leaves, it was observed that the highest chlorophyll content (30.6) relates to the application of the doses 50 Gy. The lowest chlorophyll content (7.6) relates to the application of the dose 150 Gy. There were changes in the maturity period for the two gamma rays doses (100 Gy and 150 Gy). Regarding the chemical mutagens for the dES maximum concentration 0.020 M, the values of photosynthetic pigments varied between 19.6 and 39.30, whereas for the EMS in maximum concentration 0.3%, the values of photosynthetic pigments oscillated between 14.01 and 40.80. Induced mutations in *Phaseolus vulgaris* have increased the photosynthetic chlorophyll pigments at a considerable rate. Here, additional field experiments would be needed to determine mutant lines.

***Keywords*:** chemical and physical mutagens, chlorophyll pigments, *Phaseolus Vulgaris*, mutation

1. INTRODUCTION

Mutation induction techniques are diverse, but in general they are divided into two major groups based on the type of mutagenic agents used for changes at the genetic level in chemical and physical induction techniques. Physical and chemical mutagens have been used successfully in plant breeding programs to artificially generate genetic variations for the development of new varieties with improved traits (Kodym and Afza, 2003; Ylli *et al*., 2007; Stamo *et al*., 2012; FAO / IAEA, 2018; Ylli *et al*, 2018). One of the photosynthetic pigments such as green chlorophyll in plants plays the role of a biomolecule with interest that enables the process of photosynthesis. The higher chlorophyll amount, the healthier the plant is. Therefore, the chlorophyll amount provides the researchers, agronomists and farmers with valuable diagnostic information to address nutritional and irrigation management, pesticide control, stress assessment environmental etc. (Süß *et al*., 2015).

Chlorophyll mutations and the amount of photosynthetic pigments in plants are a means to address the evolution of genetic effects as a result of mutagenic treatment (Usharani *et al*., 2015). The frequency of chlorophyll mutations in beans was found to be linearly correlated with dose. However, at high doses the frequency of mutation is reduced due to the low fertility in M1 generations. The latter occurs due to the drastic action of mutagenic treatments on cell strands resulting in high gamete and zygote sterility, leading to M1 generation mutations with a lower probability of represented by mutants in the M2 generation. Mutants obtained by induced mutagenesis show superior traits on cultivars such as higher yields due to increased resistance to biotic and abiotic factors, improved nutrient content, and other quality traits. 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).

2. MATERIALS AND METHODS

The present investigation was carried out from 2014 to 2016 involving the bean seeds of Shijak variety obtained from the National Seed and Seedling Entity which is the Genetic Bank of Albania, of the Institute of Plant Genetic Resources, Agricultural University of Tirana. Here the method of chemical and physical mutagenesis was applied to treat the seeds. The mutagens used were EMS (ethylmethanesulfanate) and dES (diethylsulfate), in three different doses each of them. Also, three different doses of gamma-radiation were applied on the bean seeds to evaluate the radiation effect in the photosynthetis pigments values. The experiment was carried out at the Mutagenesis Laboratory, in an experimental field in Fieri, Albania, and in the greenhouse of the Department of Biotechnology, Faculty of Natural Sciences, University of Tirana and in line with the IAEA (FAO / IAEA, 2006; FAO / IAEA, 2018) protocols. In the greenhouse experiments for mutagenesis in beans, the temperature and humidity were controlled, and plants were grown in pots with selected soil that provided the plants with necessary nutrients.

The gamma radiation with Cs-137 radiation source in three different doses; 50 Gy, 100 Gy and 150 Gy was applied for the treatment of the bean variety. The seeds that were subjected to irradiation were uniform, healthy, with high germination power and moisture content from 12-14%.

The seeds were kept for at least 3 days in a desiccator with 60% glycerol to balance the moisture content at 12-15% seed levels prior to radiation. The seeds were subsequently packaged in small containers adapted to the sample size in order to minimize air content. Some samples were packed in paper bags before being placed in the container to avoid contamination or the mixing of the seeds with each other. The Petri dishes or smaller containers were used for smaller seeds. The container filled with packaged samples was equipped with holders that were fixed in a standard position. Finally, the containers were placed in the radiation device in which the radiation dose was determined.

Once this process ended, the materials were kept in the laboratory for a week at temperatures varying between 20-22^{0}C, and were prepared to be planted based on the relevant schemes in the laboratory greenhouse and experimental plot in field conditions (Stamo *et al*., 2007; Jaupaj *et al*., 2013).

The chemical mutagenic treatment of materials was in line with (FAO / IAEA, 2018), and the used doses of mutagens were in line with the manual of the IAEA atomic agency (FAO 2006). Selected seeds were introduced to be inoculated into beakers filled with distilled H_2O where each ml of H_2O corresponds to one bean. After 4 hours, the distilled water was removed from the beakers where the preparatory phase took place, and the respective solutions prepared in advance for each mutagen with the following concentrations were added:

Diethylsulfate - dES

- 0.0025M 0.140ml in 600 ml distilled H_2O
- 0.005 M 0.282 ml in 600 ml distilled H_2O
- 0.010 M 0.564ml in 600 ml of distilled H_2O

Ethyl methane sulfonate - EMS

- 0.1% 0.1510 ml in 600 ml distilled H_2O
- 0.2% 1.020ml in 600 ml of distilled H_2O
- 0.3% 1.530ml in 600 ml of distilled H_2O where they stand for one hour

During each treatment, the seeds were kept for one hour. After the respective treatments for each mutagen, the seeds were rinsed for 4 hours in tap water to confirm the absence of the chemical mutagen residues with which they were treated on plant materials. Once rinsed, the seeds were placed on filter paper for about 24 hours to dry up. Once dried up, the seeds were divided to be sown in greenhouses and plots (FAO, 2011). The seeds were planted in the plot according to a randomized block, with 2 repetitions. The seeds were watered 3 times a week to prevent them from suffering from drought stress and provide the necessary moisture conditions for growth. At the same time the materials were planted in the greenhouse in two pots with two repetitions per dose (FAO, 2017).

The Figure 1 depicts the chlorophyll meter CCM-200 used to measure the amount of chlorophyll for all doses of radiation treatment. This equipment has been designed to measure chlorophyll content in plants and cultivars, and it is particularly useful for managing the nitrogen fixation programs for scientific research.

Fig. 1: The CCM-200 chlorophyll meter and the measurement of the amount of chlorophyll in the leaves.

Measurements were made for all doses of treatment by selecting stabilized leaves, which are equally exposed to the sun. The device was first switched on and calibrated. Once switched on and calibrated, the leaves (samples) were placed in the part where the device makes the measurements and the pigment value appeared on the screen after 2-3 seconds. Pigment measurements were always made in the same direction and position of the leaf and at the same distance from its main nerve. Measurements were made to determine the effect of mutagens and compare the doses with the control.

In addition, the impact of mutagens on the mutations was also investigated for genetic analysis purposes.

The present study evaluates the effects of treatments with physical and chemical mutagens to the plant *Phaseolus vulgaris* to improve its physiological features and the possible influence in shortening the flowering time in these plants under the influence of induced mutagenesis.

3. RESULTS AND DISCUSSIONS

Figure 2 depicts experiment process with the bean plants in field and in the plots in greenhouse. During the experiment it was observed a change in the levels of photosynthetic pigments measured by the CCM-200 chlorophyll meter. Experimental work continued until the M3 generation of some plant materials.

Fig.2: Bean plants during the generation M1 / M2 in the field and in pots in the greenhouse of the generation M2.

The effect of physical mutagens

The measurements were made in several different weeks and the progress of the photosynthetic pigments in the M1 / M2 band and their effects on the doses of physical radiation by comparing it with untreated control plants was investigated (Oladosu Y., *et al.*, 2016). The results depicted in the Figure 3 show that photosynthetic pigments have increased in all doses from week to week. A difference between each treatment regarding the dose applied could also be noted.

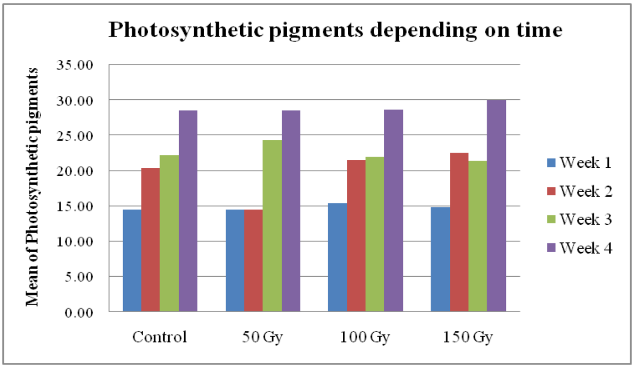

Fig. 3: The impact of physical radiation in a few weeks on the photosynthetic pigments amounts of bean plants.

After the first week the photosynthetic pigments were almost the same, and after the second week was obvious the change of photosynthetic pigments between different doses. Regarding the plants treated with 150 Gy, the photosynthetic pigments values measured in the first phases had a significant increase, something that in other phases was inhibited. The 100 Gy dose is presented with an increase in photosynthetic pigments compared to the control showing an efficacy for the effect of this dose on the amount of photosynthetic pigments. While for the 50 Gy dose, it is observed that between the two first weeks there is no difference in the values of photosynthetic pigments and then in the other two weeks a higher increase in comparison to the beginning is observed.

Photosynthetic pigments are genetically variable (Fig. 4) due to the impact of the mutagen at different doses, climatic conditions, planting location, soil type, etc. The graph plots that for both the pre-treated plants and control plants there was observed a change in the values of photosynthetic pigments. Control plants have been coming down to plants of the M2 generation. Regarding the pre-treated plants, the pigments values have increased for all three doses used.

We conclude that the impact of physical radiation doses from one generation to the next is increasing from M1 to M2 in all plants measured.

When measuring the plants pre-treated with dose 100 Gy, satisfactory results were noted during the M2 band.

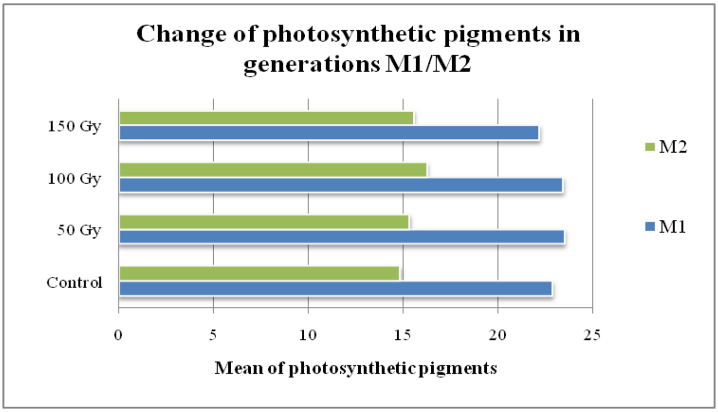

Fig.4: The impact of physical radiation on the M1 and M2 generation on the bean plant.

Investigation also involved the plants planted in two parallel plots in the greenhouse. The plants were randomly selected and photosynthetic pigments were measured via the CCM-200 chlorophyll meter. The results (Fig. 5) reported that the highest efficiency was given by the dose 100 Gy, as the stimulation of the photosynthetic apparatus of these plants was given.

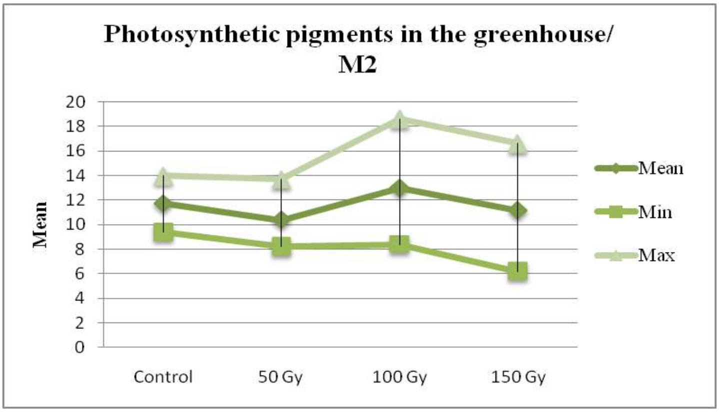

Fig. 5: The impact of physical radiation on the photosynthetic pigments on the bean plants in the greenhouse during the M2 generation.

The Table 1 reports the minimum, mean and maximum values per dose. As it could be noted, the highest values were obtained when applied the second dose showing a higher impact on the synthetic pigments of the plants planted in the greenhouse (Ibrahim R., 2010). Comparing with the control, the use with the first dose 50 Gy has led to a decrease of the mean values alike the higher dose which shows a lower value.

The effect of chemical mutagens

The M1 generation plants have been pretreated with two different chemical mutagens in three different doses such as EMS and dES. Here, the chlorophyll meter CCM-200 was also used for measurements purposes. The use of chemical mutagens in three different doses and the gamma radiation has affected the photosynthetic apparatus of plants (Table 1).

A major impact on M1 in the field compared to the control was given by the doses of EMS. The first dose appeared to be more effective than the control. The first dose of dES gave a higher variability, but an increase in photosynthetic pigments amount (Fig. 6) when applying the second dose could be slightly noted.

Table 1. The influence of chemical mutagens on photosynthetic pigments amount, maximum and minimum values

	Photosynthetic pigments amount	Max	Min
Control	29.08	38.1	22.4
dES1	27.95	41.2	16.4
dES2	28.31	36.1	19
dES3	25.26	39.3	19.6
EMS1	29.75	39.2	18.7
EMS2	26.06	36.4	14
EMS3	29.02	40.8	14.1

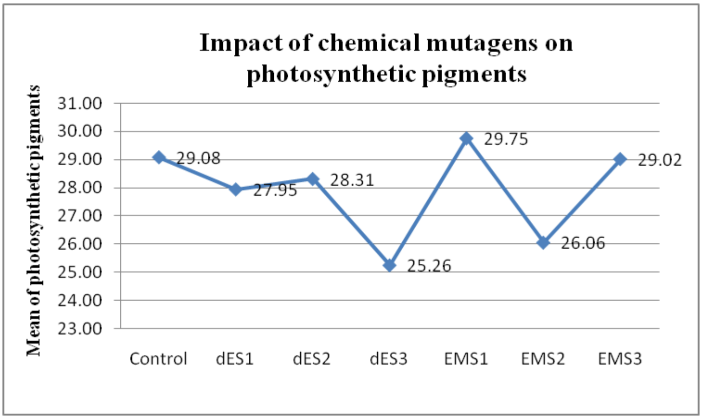

Fig. 6: The effect of chemical mutagens on photosynthetic pigments amount of bean plants during the M1 generation.

The amount of photosynthetic pigments changed from week to week, from one generation to another, and also according to the types of mutagens. The figure 7 compares the mean values of photosynthetic pigments amount during the M1 generation under the effects of chemical and physical mutagens. Here further experiments are need for accurate conclusions.

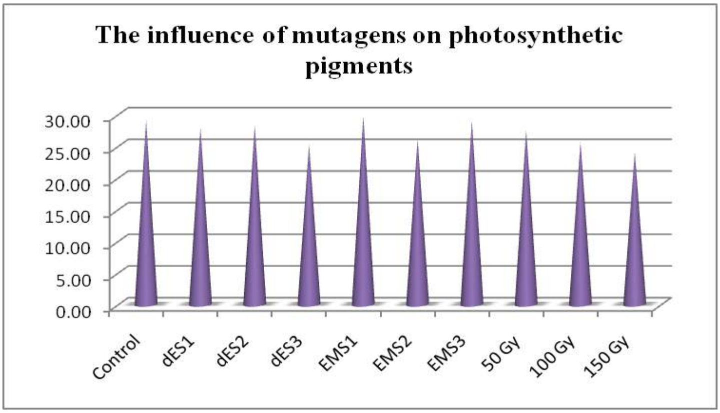

Fig. 7: Comparison of mean values of photosynthetic pigments amount during the M1 generation under the effects of chemical and physical mutagens.

Chlorophyll mutations are in themselves a phenotypic process (FAO / IAEA, 2018). The mutations appear only in the pre-treated and planted materials in generations M1, M2 and slightly in M3, and later they disappear. As chlorophyll mutations are on display for several days, they must to be constantly monitored on daily basis to be detected and evaluated. There are different types of mutations noted in the same plant regardless the treatment using the same chemical or physical mutagen. A higher number of chlorophyll mutations could be noted in the M1 band.

4. CONCLUSIONS

Bean seed-induced mutagenesis has a positive effect on the level of photosynthetic pigments, and on the occurrence of chlorophyll mutations. Since the amount of chlorophyll is an indicator of plant health, it can be said that a dose of 100 Gy radiation improves the condition of plants in the greenhouse or in the field. In addition, chemical mutagens at lower doses provided a positive influence on photosynthetic pigments. Mutations induced in *Phaseolus vulgaris* have greatly increased photosynthetic chlorophyll pigments, but further experiments to determine mutant lines are needed.

REFERENCES

FAO. 2011. Plant Mutation Breeding and Biotechnology. ISBN 978-92-5-105000.

FAO/IAEA. 2006. Mutant varieties database. Plant breeding and genetic section, Vienna, Austria.

FAO. 2017. The future of food and agriculture Trends and challenges Food and Agriculture Organization of the United Nations.

FAO/IAEA. 2018. Manual on Mutation Breeding - Third edition. Food and Agriculture Organization of the United Nations. ISBN 978-92-5-130526-3.

Jaupaj O, Stamo I, Ylli A, Bardhi N. 2013. Në përpjekje për përshtatjen e bimës së grurit me pritshmëri klimatike duke përdorur teknikat e mutagjenezës eksperimentale. *Buletini i Shkencave Natyrore,* Nr. **16**: http:/buletini.fshn.edu.al/ p.122-130.

Ibrahim, R. (2010). The malaysian nuclear agency gamma greenhouse. *Plant mutation reports. 2:50-51*

Kodym A, Afza R. 2003. Physical and chemical mutagenesis. Methods Molecular Biology.;**236**:189-204. doi: 10.1385/1-59259-413-1:189.

Usharani KS, CR. Ananda Kumar (2015). Mutagenic effects of Gamma Rays and EMS on frequency and spectrum of chlorophyll mutations in Urdbean (Vigna Mungo L. Hepper). *Indian Journal of Science and Technology,* **8(10):** 927–933.

Mba. 2013. Methodology for Physical and Chemical Mutagenic Treatments. Biotechnologies for plant mutation breeding.

Oladosu Y., Rafii M. Y., Abdullah N., Hussin G., Ramli A., Rahim H. A., Miah G., Usman M., (2016). Principle and application of plant mutagenesis in crop improvement: a revieë. *Biotechnology & biotechnological equipment, V.30, 1-16.*

Stamo I. Babani F, Ylli A. 2012. The effects of induced mutations on agronomic and physiologic parameters in cotton varieties. *AKTET.* Revistë shkencore e Institutit të Alb-Shkenca. **5** (**1**)**:** ISSN2073-2244 (33-41).

Stamo I, Ylli (Kraja) A, Dodbiba A. 2007. Induced mutations for improving production on bread and durum wheat. *AIP Journals***,** 899. Editor(s): Serkant Ali Cetin, Iskender Hikmet, Dogus University ISBN:978-0-7354-0404-5 pg. 1134-1135.

Süß A, Danner M, Obster C, Locherer M, Hank T, Richter K. 2015. Measuring Leaf Chlorophyll Content with the Konica Minolta SPAD-502Plus – Theory, Measurement, Problems, Interpretation. EnMAP Field Guides Technical Report, GFZ Data Services.

Ylli A, Xhauri Z, Bregu M. 2018. Advanced mutagenesis in decorative plants 3rd International Conference on Applied Biotechnology (3rd ICAB) Proceeding's Book, November 23 – 24, 2018, pg. 161 -168 ISBN: 978-9928-237-36-1.