CHLOROPHYL MUTATIONS IN HARICOT PLANTS TREATED WITH MUTAGENS

***MALVINA KODHELAJ, ARJANA YLLI**

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

e-mail: malvinakarcini@yahoo.com

Abstract

Beans (*Phaseolus vulgaris*) represent a high genetic variability and important for agricultural production. Through techniques of induced mutation on bean seeds was tried to shorten the time of flowering so that to eliminate abortion of flowers in this period. Bean seeds Shijak variety are irradiated with gamma radiation of Cs-137 with three doses, and are treated with chemical mutagen dES and EMS also in three different doses and control. Results obtained in the first generation of mutant M1 indicate changes compared to control for the both treatments. Chlorophyll mutations were identified in all the vases treated with mutagens or radiation in advance. Some fair-colored stains were noticed in the leaves as a result of mutagenic activity. The dosage of 100 Gy is advantageous because it was the first to give flower after only seven weeks of being planted. Whereas, chlorophyll mutation with albine features are noticed in plants with a dosage of 150 Gy. Chlorophyll mutations such as whitening, depigmentation, and leaf serration are observed in plants treated with radiation at a dosage of 100 Gy. Depigmentation and leaf malformation is observed in haricot beans radiated with a dosage of 50 Gy.

Key words: Chemical mutagen, Chlorophyll mutations, Gamma irradiation, Mutation.

Përmbledhje

Fasulja (*Phaseolus vulgaris*) shfaq një variabilitet të lartë gjenetik dhe me rëndësi për prodhimin bujqësor. Nëpërmjet teknikave të mutagjenezës së induktuar në farat e fasuleve, është tentuar shkurtimi i kohës së lulëzimit në mënyrë që të eliminohet abortimi i luleve në këtë periudhë. Farat e fasules Varieteti Shijak rrezatohen me rrezatim gama Cs-137 me tre doza krahasuar me kontrollin, dhe trajtohet me mutagjen kimik dES dhe EMS në tre doza krahasuar me kontrollin. Rezultatet e marra në brezninë e parë mutante M1 tregojnë ndryshime në krahasim me kontrollin për të dy trajtimet. Mutacionet klorofiljane u vërejtën në të gjitha vazot e trajtuara me mutagjenë dhe me rrezatim. Disa njolla me ngjyrë të çelur u vunë re në gjethe si rezultat i veprimit të mutagjenit. . Në trajtimin me dozën prej 100 Gy bima lidhi lule më herët se në trajtimet e tjera, ka një përparësi sepse ishte e para që lidhi lule vetëm pas 7 javësh nga momenti i mbjelljes. Kjo tregon që me stabilizimin e breznive dhe me studime të mëtejshme mund të arrihet në një pjekje të hershme të fasuleve. Ndërsa në dozën 150 Gy vihen re mutacione klorofiljane me tipare albine, zbardhje dhe depigmentime të gjetheve. Mutacionet klorofiljane si zbardhje, depigmentim dhe dhëmbëzim i gjetheve vërehen në bimët e trajtuara me rrezatim në dozën 100 Gy. Në fasulet e rrezatuara me dozen 50 Gy vërehen disa mutacione klorofiljane si depigmentime dhe shformime te gjetheve.

Fjalët kyçe: Mutagjenë kimik, Mutacione klorofiljane, Rrezatim gama, Matacione.

Introduction

Mutations are heritable changes that occur in the genetic material of living organisms. These changes occur for various reasons and may be natural or induced. They can be recognized as phenotypic variants at different stages in the life cycle, but the primary effects are genetic (Lundqvist, Franckowiak and Forster, 2012). Phenotypic selection is a fundamental part of plant breeding and therefore, there has been a natural link between mutant phenotypes and their selection by plant breeders, which traditionally has been based on phenotypic selection (FAO/IAEA 2018).

It has been several years now that the usage of induced mutations with various mutagenic agents has tuned into one of the main ways of creating new cultivars with improved features compared to parent material. Mutagenic induced technology has been recently accepted as a valuable additional tool in creating improved cultivars in agriculture (FAO/IAEA 2018). Induced mutations have been used to improve major crops such as wheat, rice, barley, cotton, peanut and cowpea, which are seed propagated (Buschmann *et al*. 1988). Various mutagenic agents are used to induce favorable mutations at high frequency that include ionizing radiation and chemical mutagens (Ahloowalia *et. Al*, 2001). Many physical and chemical mutagens have been used for induction of useful mutants in a number of crops. Mutation breeding has been extensively followed in other vegetable crops like tomato, chilies etc, (Gustafsson, 1986). Chemical mutagenesis is a simple approach to create mutation in plants for their improvement of potential agronomic traits. Mutations are the tools and being used to study the nature and function of genes which are the building blocks and basis of plant growth and development (Adamu *et al*. 2007).

The haricot bean in our country is considered as one of the main leguminous plants. It (*Phaseolus vulgaris*) is a highly genetically variable species and very important for agricultural produce. The climatic changes of recent years have caused a considerable decrease in its production. The evidence of haricot decrease has resulted in conducting studies that aim to reduce the rate loss. One of the main identified issues is the abortion of the haricot bean flower, that is, pods' inability to link because of high temperatures and seasonal droughts. Induced mutagenic techniques have been used in the haricot bean seeds to shorten the blooming period in order to reduce the abortion of haricot bean flower in such periods. Chlorophyll mutations have been used as genetic markers in physiologic and biochemical studies. In origin, chlorophyll mutations are caused mainly because of mutations in both genes, which are responsible for photosynthetic pigment synthesis (FAO 2017). They are the most frequent and easily identified mutations in M2 generation. Up to now, 16 types of chlorophyll mutagens are known and found in all varieties and mutagenic treatments. In 1940, the Swedish scientist Gustaffson provided preliminary knowledge on the usage of these mutations in order to study and explain the effectiveness of physic mutagens in relation to ionizing radiation. Chlorophyll mutations are classified in 9 different groups (Gustafsson, 1986). This system is especially appropriate for barley, but it is also useful for chlorophyll mutations in general. Chlorophyll mutations make themselves a phenotypic process (Sestak, 1971). They exist in untreated material, in M1/M2 generations and a little bit in M3 generation but not in later generations.

Materials and methods

We choose as material the seeds as they are easier to treat and it's easier to control the mutagen dose. We choose Shijak variety, collected from Kruja and Lushnja.

Physical mutagens gamma rays of **Cs-137**, and most of materials were irradiated with three different doses and power of dose 485.6 R/min.). M0 the number of seeds irradiated is 1000 seeds per which dose 50 Gy, 100 Gy and 150 Gy. The radiation dose 50 Gy, 100 Gy, 150 Gy are calculated based on the IAEA protocols and are applied in IANP facility in Tirana. These three doses could be compared with the untreated parenting materials.

Irradiated seeds were planted in field into the experimental parcel in Fieri and in the Institute of Applied Nuclear Physics greenhouse.

In the field was used a randomized complete block with split plots (4m x 0,8m) and four replications while in the greenhouse were planted 2 replications.

Chemical mutagens used: dES (diethylsalphat) with 0.010 M dose, 0.015 M, 0.020 M, and EMS (etylmethansulphonat) in doses of 0.050%; 0.075%; 0.10%; 0.15% respectively.

All treatment was done according the methods and using the IAEA Manual about the ways of treating plant materials (FAO 2011& FAO/IAEA 1977 & FAO/IAEA 2018).

Results and discussions

In addition, various mutations have been observed under the influence of mutagens. Mutant materials have clearly shown chlorophyll mutations in their leaves. The observations in the first generation mutant to assess the phenotypic characteristics and possible mutations in this generation and the possibility of comparing the adjustment in some areas of the country (Gonzalez, *et al*. 2002). After planting in the field is made continuous assessment for the identification of species or specific herbs with improved properties under the influence of mutagenic substances by comparing them with untreated parental materials. In M1 generation we accumulated all plant material by the literature and made recommendations and their assessment in the laboratory, for all doses used, including control (Mak, *et al.* 1996).

Materials were observed and evaluated for chlorophyll mutations in the M1 generation as well as in the following year in the M2 generation.

Chlorophyll mutations type maculate appear with chlorophyll destruction in some areas as seen in Fig. 2. Some white spots appear and in these areas where the spots have appeared we have pigment destruction and consequently the leaf turns white in some parts of it. This type of mutation is more common in 100 Gy treatment doses (Fig. 1). In the plants that were found in the greenhouse it is at the rate of 5% in the generation M1 and in the following year M2 at the rate of 2%.

During the period of vegetation, data on phenological phases (sprouting, branching, blooming and maturing) have been recorded. In the flowering phase there was a dose of 100 Gy in its acceleration of 5 days compared to the control. While in the 150 Gy dose there was a flower bloom with 3 days. This indicates that the 100 Gy dose for the bean plant is optimal for accelerating the flowering of *Phaseolus vulgaris* flowers. The assessment and measurement is done in a laboratory for the pod and the grain (Gonzalez, *et al*. 2002). Evaluation of all physiologic indicators is realized based on IPGR descriptions. Chlorophyll mutations were identified in all the vases treated with mutagens or radiation in advance. Some fair-colored stains were noticed in the leaves as a result of mutagenic activity.

Moreover, white-colored stains appeared and the pigments around that area were destroyed. As a result, some parts of the leaf turned white. Mutations have brought many changes in the leaf's morphology. It is easy to notice tucked leaves in some of these parts. Thus, in general we can say that every mutagen has caused mutations in plants, some of which are chlorophyll mutations and others not. It is interesting that in the same plant we observed different types of mutations although the treatment was the same and done with the same mutations (McCallum, *et al*. 2000). Besides other analysed differences, radiation causes change in the color of the flower. The normal color of the haricot flower of Shijak variety is white (Fig. 5). After treating them with radiation, the color changed into yellow. Another change occurs in the flower's placement.

Figure 1. Chlorophyll mutations in irradiated beans plants with 150 Gy dose M1 generation.

Figure 2. Chlorophyll mutations in irradiated beans plants dose with 100 Gy in M1 generation.

The dosage of 100 Gy is advantageous because it was the first to give flower after only seven weeks of being planted (Fig. 4). This shows that generations' stability and further studies can result in an earlier ripeness of the haricot. Wheras, chlorophyll mutation with albine features are noticed in plants with a dosage of 150 Gy. Also, a decrease in the ability to sprout is noticed in haricot seeds. Chlorophyll mutations, twisted, malformed and serrated leaves are noticed in M1 plants (Wani, *et al.* 2017). There are 5 chlorophyll mutations within the same plant. Being temporary phenotypic features the chlorophyll mutations are observed on weekly bases not on daily bases (Paterson, *et al.*, 2009).

Figure 3. Chlorophyll mutations in M2 generations in greenhouse materials.

Tigrin type mutations were observed in plants treated with the 150 Gy dose. These mutations had a higher frequency in the M1 generation going up to 6% (Fig 2). Chlorina - we notice that in the leaves have appeared some light-colored spots as a result of the action of the mutagen. Viridis - heterogeneous group characterized by green. They appear in the same plant with lighter colored leaves than the others. We also notice changes in the nerves in the leaves that are adjacent to each other, so the mutagen used in this plant has brought different changes in the leaves of the same plant (Fig 3).

In this plant there are clear changes that have occurred in its 3 leaves. Nerve changes have occurred; the leaves in some parts have been collected and even suffered damage. And why the leaves are of the same plant and are treated with the same mutagen dose the differences are not the same. We find different chlorophyll mutations inside the same plant.

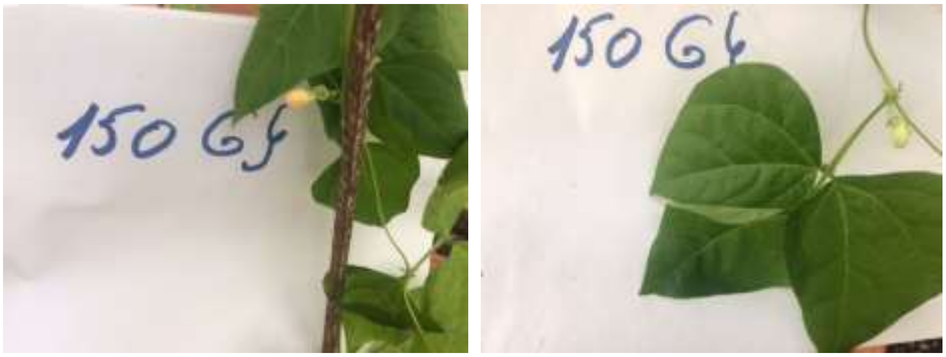


Figure 4. Mutation of floral color of irradiated beans plants dose of 150 Gy in greenhouse.

Figure 5. Mutation of floral color of irradiated plants, planted in the experimental field.

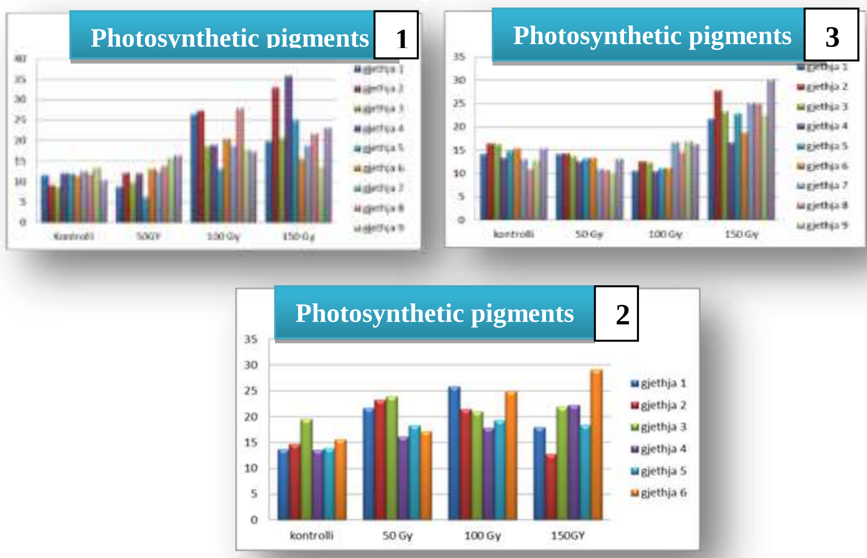
Chlorophyll mutations such as whitening, depigmentation, and leaf serration are observed in plants treated with radiation at a dosage of 100 Gy. Chlorophyll mutations such as depigmentation, whitening, twisted, malformed and serrated leaves, i.e tigrina and viridis mutations are observed in 3/4 of haricot beans (Fig 3). Depigmentation and leaf malformation is observed in haricot beans radiated with a dosage of 50 Gy.

Figure 6. Radiated plants have three pods compared to control plants that have only one pod.

Besides chlorophyll mutations in plants treated with radiation, another feature has appeared because of mutations such as the color of the flower and the number of beans. In plants with radiation dosage of 150 Gy, the color of flowers in control plants is white whereas in mutated plants is yellow. It is observed that mutagenesis has shortened the blooming period and radiated plants have three beans compared to control plants that have only one bean.

Since the chlorophyll amount is an indicator of plant health, we can say that 100 Gy irradiation dose improves the condition of plants in the greenhouse or on the field. Photosynthetic pigments differ from one generation to another due to the action of mutagens and climatic conditions of the area where the seeds are planted (Mahamune, *et al.* 2012, Rajarajan, *et al.* 2014). Based on the data obtained from the last year (2013-2014) we compared the photosynthesis pigments between M1 and M2 generations. Since the chlorophyll amount is an indicator of plant health, we can say that 100 Gy irradiation dose improves the condition of plants in the greenhouse or on the field.

The growth of legumes to a greater length is clearly discernible when the plant has been treated with the mutagen compared to what is seen in the untreated control where only a formed pod is observed. In our material created under the influence of mutagens the beans are whole. As a result of the action of the mutagen, the flower growth is clearly visible in the figure compared to the control.



Graphic 1. The measurement of the photosynthetic pigments in materials planted in the 1-laboratory, 2- greenhouse and 3-field.

From the measurements of chlorophyll pigments in the leaves of plants grown in different environments large differences are observed (Graf.1). Treatment with dose of 100 Gy is the one that has given stability in the values of chlorophyll pigments.

Conclusions

In our plant materials a high frequency of chlorophyll mutations was observed up to 6% of the analyzed population. The dose of radiation used 150 Gy was the one that gave numerous mutations such as Albine, Tigrine and Viride cases. In these materials the highest frequency in their occurrence had the chlorophyll mutations Tigrine.

In radiation treatments the 100 Gy dose had less variety of mutations and Striata and Maculata appeared.

Radiation-induced mutagenesis in the M1 and M2 generations, in all three treated doses, we observed changes in bean flower color from white to yellow.

At the time of flowering there was a shortening of the flowering time with 5 days of opening earlier at the dose of 100 Gy.

The number of legumes bound in the mutant material was higher compared to the control. Regarding pod characteristics, the best results were obtained by 50 Gy dose because it increased the performance for the measured features in the pod.

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