PHASEOLUS VULGARIS TREATED WITH PHYSICAL MUTAGENS AND ASSESSMENT OF CHLOROPHYLL MUTATIONS AND SYMBIOTIC BACTERIA

Arjana YLLI, Selvina ÇOLLAKU and Emanuela HAJDERAJ
Biotechnology Department, Faculty of Natural Science,
University of Tirana, Albania

ABSTRACT
The bean (*Phaseolus vulgaris*) represents a crop source for food and sustainable agriculture in the world. Unfortunately, climate change has a significant impact on their yield. Induced physical mutagenesis has a long-term remarkable potential of improving plant material with regard to their qualitative and quantitative production characteristics. The present paper investigates the seeds of *Phaseolus vulgaris*, a cultivar registered in the Albanian Genetic Bank. The seeds are treated with physical mutagens, gamma radiation Cs-137, in three different doses (50 Gy, 100 Gy and 150 Gy). The present investigation informs about the planting of these materials in three different environments; greenhouse, laboratories and experimental field. *Rhizobia* bacterium plays a significant role in provision of agricultural ecosystem services due to their ability to form symbiotic association with a wide range of leguminous plants that results in biological nitrogen fixation. The bacteria have been microscopically identified. Once identified, they were isolated from the root nodules of beans which were treated with physical mutagens, and young rhizobia cultures were grown in YEMA medium. Various mutations were reported throughout the experiment. In laboratory and greenhouse plants, some types of chlorophyll mutations appeared to be dependent upon the radiation dose. In addition, striata, maculate, chlorine and tigrine, types of chlorophyll mutations reported changes in the amount of chlorophyll. Compared to one year ago with M_1 generation, albviridis is the only chlorophyll mutation not occurring in the present investigation. Nodules are smaller in size due to mutagenesis. Stero-microscope images show nodules being green and brown in color due to the presence of chlorophyll in the cortical region of the nodule and the presence of leghemoglobin protein in the nodule, respectively.
Keywords: *Phaseolus vulgaris, Physical mutagens, Rhizobium*

1. INTRODUCTION

Ulukapi and Ozmen (2019) stated that common bean is an important crop for food and sustainable development in agriculture. *Phaseolus vulgaris* also known as the bean belongs to the group of leguminous plants, and is widely found across the country. The bean is considered to be one of the most important products in the leguminous group (Ylli *et al*, 2019; Kodhelaj and Ylli 2021). *Phaseolus vulgaris* is very important for agricultural production due to its high genetic variability. Climate changes unavoidably project decrease in vegetables and legumes' yields (Heinemann *et al.*, 2017; Kodhelaj and Ylli 2021). Bean production decrease has led to many studies to power the elimination of performance losses in field. One of the problems identified is the abortion of flowers of the beans as legumes do not survive due to high temperatures (Ylli *et al*, 2013; Kodhelaj and Ylli 2017). As the decrease in bean yield results from a low percentage of fruit production from flowers when drought occurring during flowering and from embryos abortion during the pod-forming stage, continuous efforts have been made to adapt legumes to new environmental conditions. Induced mutations have been for a long time a means to address the new cultivars with improved features when compared to the parents. Induced mutagenesis technology has been recently recognized as a valuable additional tool to create improved cultivars in agriculture (FAO/IAEA, 2018). Induced mutation by using mutagen is appropriate for genetic variability (Kshirsagar *et al.*, 2014). The experience has shown that mutagenesis is one of the most important direction for the creation of new crop variety especially at leguminous. Physical mutagens are generally preferred by reason of being convenient, easily reproducibility, and user environment-friendly method (Çelik and Atak, 2017). Bacterial nitrogen fixation is the biological process by which atmospheric nitrogen is uptake by bacteroid located in plant root nodules and converted into ammonium through the enzymatic activity of nitrogenase. In practice, this biological process serves as a natural form of fertilization and its optimization has significant implications in sustainable agricultural programs (Resendis-Antonio *et al*, 2011). Rhizobia bacteria play a significant role in provision of the agricultural ecosystem services due to their ability to form symbiotic association with a wide range of leguminous plants that results in biological nitrogen fixation (Koskey *et al*, 2018). The infection of legume roots by rhizobia, leading to the formation of nitrogen-fixing nodules, is a clonal event and each individual bacterium that initiates an infection can grow rapidly (Frederix *et al*, 2014). Lateral gene transfer of specific symbiosis genes within rhizobia genera is an important mechanism allowing legumes to form symbioses with rhizobia adapted to particular soils. Rhizobia-legume symbiosis is a host-specific association and enhances the need to determine the strains and the diversity of rhizobia associated with specific type of legume for better exploitation of the benefits associated with the rhizobia biofertilizers (Batista 2015; Koskey *et al*, 2018). As strain-specific legume rhizobia symbioses can develop in particular habitats (Andrews and Andrews 2017), the present paper aims to identify the presence of rhizobium bacteria in plants treated with mutagenesis and grown in different environments.

2. MATERIALS AND METHODS

The seeds of *Phaseolus vulgaris*, a cultivar registered in the Albanian Genetic Bank, are in the present investigation used. This cultivar is mainly planted in Fieri and Lushnja regions. The seeds of *Phaseolus vulgaris* are treated with physical mutagens, gamma radiation of radiation source Cs-137, in three doses of 50 Gy, 100 Gy and 150 Gy compared to the control at the Institute of Nuclear Physics, University of Tirana, Albania. Materials are planted in different environments; greenhouse, laboratories and experimental field in the outskirts of Tirana. *Rhizobium* was microscopically identified and quantified, and isolated from bean root nodules. Evaluation required plants with nodules for isolation purposes.

Rhizobium was isolated from bean root nodules treated with physical mutagens. Isolation of rhizobium was carried following the standard protocols as described in (Somasegaran and Hoben 1994). Healthy and undamaged root nodules of *Phaseolus Vulgaris* treated with physical mutagens from various environments; greenhouse, laboratories and experimental field were selected and used for the isolation of *rhizobia* bacteria. The nodules were washed and sterilized, and the 10 healthy and undamaged nodules were collected from each plant. The nodules were detached from the root by cutting 0.5 cm on each side of the node. Undamaged nodules were immersed for 5-10 seconds in 95% ethanol, and subsequently transferred to a 3% solution of sodium hypochlorite, for 4 minutes.

To make the nodule preparation, a loop of the nodule suspension is placed on a mannitol agar (YEMA) plate with yeast extract and then this material is incubated in the dark at 28ºC for 3-5 days. Maked wet mounts of the cultures were provided and examined under the phase contrast microscope, and the standard materials microbiological techniques described by (Somasegaran and Hoben, 1994) were employed. All the isolates were characterized by morphological parameters such as colony size, shape and color. Other tests that were carried out included gram staining where young pure isolates (5 days old) cultured on YEMA were smeared on clean microscope slides (Beck *et al*, 1993; Koskey *et al*, 2018).

3. RESULTS

There are numerous chlorophyll mutations reported which could be classified into 9 groups based on the Gustafsson's method. In the laboratory and greenhouse plants, some types of chlorophyll mutations appeared to be related to the radiation dose. So, *Phaseolus Vulgaris* had unifoliate and trifoliate leaves, different in size. Seedlings grown from seeds irradiated with 50 Gy gamma dose and planted in Petri dishes were observed to grow faster. The Figure 1 depicts the plant with the chlorophyll mutation albviridis induced by 300 Gy gamma rays. Seed germination was reported in seeds irradiated with 150 Gy gamma dose. The striata mutation was characterized by pronounced longitudinal stripes, maculate stained throughout the leaf due to the destruction of the chlorophyll. The chlorine mutation was characterized by larger stains which had light color. The tigrine mutation was characterized by pigments destruction with the narrow transverse stripes which were yellow in color (Kodhelaj and Ylli 2017; Kodhelaj and Ylli 2021).

These types of mutations occur in all materials obtained after treatments. There were changes in chlorophyll pigment under natural conditions reported. Here, we could mention maculate, chlorine and viridis mutations. The latter is characterized by a light green. There are changes related to the morphology of bifoliate and tetra foliate leaves and the concentration of pigments causing chlorophyll mutations such as chlorine, viridis, maculate, xantha and albviridis if compared to one year ago with M1 generation (Figure 1). The latter is characterized by the green leaf and its most discolored tip. It is the only mutation that has not appeared in the present investigation (Gustafsson 1986). Chlorophyll mutations are the most frequent mutations and easily identifiable in the M2 generation.

Fig. 1: Different tipe of Chlorophyll mutation in *Phaseolus vulgaris* treated with physical mutagens in M1 generation.

Stress caused by high temperatures develops a deeper root system that absorbs more water, but the growth of the root system decreases the productivity. Here, either multiplication of roots hair for a more effective use of the same biomass or stimulation of a higher acid production in roots to keep the same production level or increasing it would be necessary.

Figures 2-4 show the nodes observed in the plants planted in Petri dishes for the materials irradiated with three different doses and their untreated control. In the Petri dishes the plant growth is more restricted, and the root system less developed. The Stereomiscroscope method was applied to measure the root 12 and 15 days after germination. The root system for bean plants treated with three doses of radiation are compared to the control, and the results show changes throughout their development process.

Fig. 2: Control plant growth in laboratories.

Fig. 3: Plant treated with 50GY doses.

Fig. 4: Plant treated with the dose 100 GY.

Fig. 5: Plant treated with the dose 150GY.

4. DISCUSSION

Leguminous plants have the capacity of fixing nitrogen in their roots. Nodules are smaller in size and amount than the control due to mutagenesis. The stereomicroscope images show green and brown nodules. The greenhouse and soil plants showed the presence of green nodules. These nodules are close to the earth's surface, and the green color proves the presence of chlorophyll in the cortical region of the nodule. The data obtained from the microscope show that the rhizobium bacteria had bacillus and rod-shaped form, gram negative and their number in plants irradiated with 50 Gy gamma doses and 100 Gy gamma doses was smaller than in control plants (Figure 6).

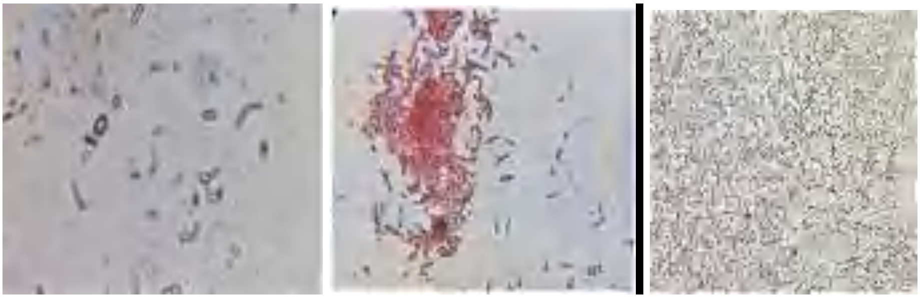

Fig.6: Rhizobium from plant treated with doses 50 Gy, 100 Gy and control under the microscope**.**

Mutation breeding can be used non only to induce mutations but also to promote genetic recombination to increase mutations' frequency (FAO/IAEA, 2018). Induced mutation is an important tool for producing genetic variation (Chusreeaeom and Khamsuk, 2019). Chlorophyll mutations appeared dependent upon radiation dose. The types of mutations observed during the experiment are macromutations in different degrees.This is most pronounced in the case of the xantha, viridis and alboviridis types and less pronounced in the case of the albina and tiger types (Gustafson 1938; Gustafsson 1986; FAO/IAEA, 2018).

The bean plants planted in the greenhouse showed undefined mutations accompanied by changes in the shape of the leaves, their wrinkles and color. Albine mutations report lethal mutation characterized by entirely white leaves of seedlings; seedlings survived for 10-12 days after germination. The bean plants undergoing the chlorine mutations have light green leaves. In addition, although most of the seedlings died within 20 days, few vigorous plants survived and were late in maturity. During the maculata mutation, seedlings showed either yellow or white dots on leaves, and these mutants survived till maturity producing few seeds. The xantha mutations displayed on the *Phaseolus Vulgaris* leaves gave the typical characteristics of light green color of leaves, most of the seedlings died within 20 days. In the leaves of our plants alboviridis mutations appear different colors at the leaf base and leaf tip. These mutations in the greenhouse were quite noticeable.

Chlorophyll mutations are frequently employed for the determination of mutagenic potency in inducing genetic variability as they are vital indicators in the assessment of induced genetic changes of mutagenized population (Raina and Khan. 2020). The green color appears due to the development of chlorophyll in the cortical region of the nodule, while the brown color proves the presence of the leghemoglobin protein in the nodule (Somasegaran and Hoben, 1994).

The root system is an important factor for plant productivity in leguminous plants. The length of the roots was measured 12 and 15 days after germination. The presence of a developed root system was observed in the three different environments where the mutagenized bean plants were planted. The plants planted in the ground are healthier and have rounded nodules as they grow under favorable conditions due to their natural environment. Gram staining results and growth on YEMA media, confirmed preliminary the standard morpho-cultural characteristics of *Rhizobium* species that nodulate with *P. vulgaris* (Koskey *et al*, 2018). Strain-specific legume rhizobia symbioses can develop in particular habitats (Andrews and Andrews 2017). The presence of the rhizobium bacterium in mutagen-treated plants also confirms that these plants have a great ability to fix the nitrogen.

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