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GAMMA-INDUCED MORPHOLOGICAL VARIATION AND REPRODUCTIVE STABILITY IN COMMON BEAN (*PHASEOLUS VULGARIS* L.) UNDER THE AGROECOLOGICAL CONDITIONS OF BOSNIA AND HERZEGOVINA

Mirela Kajkut Zeljković[1*], Sonja Umićević[1], David Ducanović[1], Marina Antić[1], Predrag Ilić[1], Tanja Krmpot[2], Sunčica Bodružić[1], Vida Todorović[2], Jelena Nikitović[1], Nikolina Đekić[2], Mirjana Jankulovska[3]

[1]University of Banja Luka, Institute of Genetic Resources, Banja Luka, Bosnia and Herzegovina
[2]University of Banja Luka, Faculty of Agriculture, Banja Luka, Bosnia and Herzegovina
[3]Ss. Cyril and Methodius University in Skopje, Faculty of Agricultural Sciences and Food-Skopje, Republic of North Macedonia
[*]corresponding author: mirela.kajkut@igr.unibl.org

ABSTRACT

The present study aimed to induce and evaluate gamma-induced morphological variation and reproductive stability in common bean (*Phaseolus vulgaris* L.) under the agroecological conditions of Bosnia and Herzegovina. A total of 5145 dry seeds of the accession “niska boranija” from the Gene Bank of the Republic of Srpska were exposed to two gamma radiation doses (80 Gy and 200 Gy) at the IAEA laboratories in Seibersdorf. Mutant generations were advanced from M1 to M4 under open-field conditions. Phenotypic evaluation revealed stable morphological variation from the M2 generation onward. Flower colour mutation from white to violet was observed in 7.25% of evaluated M3 plants and remained stable in subsequent generations. Additional heritable changes included alterations in seed coat colour (yellow to greenish) and modifications of plant architecture, particularly the transition from determinate dwarf to climbing growth habit. In M4 generation, 79.66% of mutant lines maintained reproductive capacity, confirming the stability and usability of induced mutations. The 80 Gy treatment proved more effective in generating viable and phenotypically stable mutants, whereas 200 Gy caused stronger growth reduction and lower overall viability. This study demonstrates the successful application of gamma induced mutagenesis in common bean under the agroecological conditions of Bosnia and Herzegovina. The obtained mutant lines represent a valuable genetic resource for future breeding programs targeting yield improvement, drought tolerance and climate resilience.

Keywords: *Phaseolus vulgaris* L., gamma irradiation, induced mutagenesis, morphological variation, mutation breeding, reproductive stability

INTRODUCTION

Common bean (*Phaseolus vulgaris* L.) represents the most extensively cultivated grain legume worldwide and ranks third after soybean and peanuts when oilseed and grain legumes are considered together (Myers and Kmiecik, 2017). Common bean is grown on more than 33 million hectares, with the production of over 30 million tons per year globally (FAO STAT, 2024). Common beans are a major plant-based source of dietary protein, rich in storage proteins (mainly globulins and albumins), high in essential minerals such as iron, zinc, copper, phosphorus and aluminium, and contain bioactive compounds associated with the prevention and modulation of chronic diseases, including obesity, diabetes, cancer, and coronary heart disease (Murube et al., 2021).

Common bean production is constrained by numerous biotic and abiotic factors, particularly diseases and stresses such as drought, soil compaction, low soil fertility, and high temperatures, which collectively create a substantial gap between actual and potential yields and result in significant economic losses for farmers each year (Sofkova-Bobcheva et al., 2021). The available genetic variability within the cultivated gene pool has been exploited to the maximum level of productivity in common bean, necessitating the creation of additional variations through mutations (Kumar et al., 2021). Bean breeding programs based on mutagenesis to increase genetic diversity are commonly applied nowadays (Tomlekova, 2010; Tomlekova, 2016). These efforts have led to the development of new bean mutant varieties with high productivity, broad environmental adaptability, improved nutritional quality, and enhanced disease resistance, thereby reducing production costs, increasing product quality, and promoting environmentally sustainable cultivation (Tomlekova et al., 2024).

To date, a wide range of mutants have been developed and officially recorded in the Mutant Variety Database (MVD), maintained by the Food and Agriculture Organization of the United Nations in collaboration with the International Atomic Energy Agency; currently, the database documents 3502 mutant varieties, of which 57 correspond to common bean. In the research on common bean mutantc, Ylli and Kodhelaj (2023) reported changes in germination, chlorophyll content, pod and seed traits, and root development, with lower gamma doses (50 Gy) and lower concentrations of ethyl methanesulfonate producing the most favourable agronomic outcomes. The same authors demonstrated that the type and dose of induced mutagenesis, including gamma radiation and ethyl methanesulfonate, significantly affect phenotypic variability and yield in the Shijak variety of common bean, with lower gamma doses enhancing yield but higher doses inducing greater physiological stress (Kodhelaj and Ylli, 2017). Similarly, Kumar et al. (2021) revealed that germination percentage, root and shoot length along with number of leaves, decreased progressively with increasing doses of gamma rays and ethyl methanesulfonate. Furthermore, Sofkova-Bobcheva et al. (2021) showed that ethyl methanesulfonate treatment of common bean seeds induced extensive morphological variation across M2 and M3 generations, affecting growth habit, internode and branch characteristics, flower and pod coloration, as well as seed size, shape, and colour. In adition, sodium azide treatment in *Phaseolus vulgaris* var. Sheena induced leaf morphological variations, chlorophyll mutations, and modifications in reproductive traits, without negatively affecting plant survival to maturity (Shaikh et al., 2019). In faba bean, Khursheed et al. (2019) reported the isolation of diverse morphological mutants in the M2 generation, encompassing variations in plant height, growth habit, leaf morphology, flower characteristics, and pod traits. Collectively, these studies highlight the effectiveness of induced mutations in generating heritable morphological diversity that can be exploited in legume breeding programs.

No application of induced mutagenesis using gamma irradiation has yet been reported in Bosnia and Herzegovina. Therefore, the objective of this study was to induce and establish the first mutant lines of common bean (*Phaseolus vulgaris* L.) using induced mutagenesis, aiming to generate novel genetic variability that can be utilized in future evaluations of drought tolerance, yield potential, and adaptability under local agroecological conditions.

MATERIAL AND METHODS

Common bean (*Phaseolus vulgaris* L.) seeds were obtained from the Gene Bank of the Republic of Srpska, Bosnia and Herzegovina. The accession "niska boranija" is characterized by a determinate dwarf growth habit and white flowers. The seeds displayed uniform morphological characteristics, with an average seed size of 14.1 × 5.6 × 7.0 mm (length × thickness × width), a 100-seed weight of 31.72 g, a cuboid seed shape, and a yellow seed coat without secondary coloration.

A total of 5145 dry, healthy seeds were subjected to gamma irradiation to induce genetic variability. Gamma ray treatments were performed at the Plant Breeding and Genetics Laboratory, Seibersdorf, International Atomic Energy Agency (IAEA), using a gamma radiation source. Seeds were irradiated with two different doses in order to evaluate dose dependent mutagenic effects: 3430 seeds were treated with a moderate dose of 80 Gy, while 1715 seeds were exposed to a higher dose of 200 Gy. The selected doses were determined based on previous studies demonstrating their effectiveness in inducing mutations while maintaining acceptable levels of seed viability in common bean and other grain legumes (Tomlekova, 2010; Spenser Lopes et al., 2018; Kumar et al., 2021). Following irradiation, treated seeds were sown under open field conditions to establish the M1 generation. Individual M1 plants were grown to maturity and harvested separately. Their progeny was advanced through subsequent generations using the pedigree method. Field sowing was conducted in 2023 to obtain the M2 generation, in 2024 for the M3 generation, and in 2025 for the M4 generation. Advancing generations enabled the fixation and stabilization of induced mutations, as phenotypic variation becomes more clearly expressed and heritable from the M2 generation onward (Spenser Lopes et al., 2018). Throughout the generational advancement process, plants were systematically evaluated for survival rate, growth performance, and visible morphological variation. Particular attention was given to deviations in plant architecture, leaf morphology, flowering characteristics, and pod and seed traits.

RESULTS AND DISCUSSION

Gamma irradiation induced stable phenotypic variation in the common bean accession "niska boranija". Morphological changes were observed from the M2 generation onward and remained stable through the M3 and M4 generations, indicating fixation of induced mutations.

Plant survival and generational advancement

Seeds irradiated with both 80 Gy and 200 Gy doses germinated and produced viable plants that could be advanced to subsequent generations. However, plants originating from the 200 Gy treatment showed reduced growth and lower overall viability compared with those treated with 80 Gy. Despite this, sufficient numbers of plants survived to allow advancement up to the M4 generation. These observations are in agreement with previous studies reporting increased physiological stress and reduced survival at higher gamma irradiation doses (Kodhelaj and Ylli, 2017; Kumar et al., 2021).

Flower colour variation and frequency

Flower colour mutation was clearly expressed in the M3 generation. Out of 193 evaluated M3 plants, 14 plants (7.25%) exhibited a change in flower colour from the original white to violet (Table 1, Figure 1). This trait remained stable in subsequent generations, confirming the heritable nature of the induced mutation. Flower colour mutations are commonly reported in mutagenesis studies and represent reliable indicators of successful genetic alteration due to their simple genetic control and high visual detectability (Shaikh et al., 2019; Ylli and Kodhelaj, 2023).

Table 1. Frequency of flower colour variation in the M3 generation

Flower colour	Number of plants	Frequency (%)
White (control type)	179	92.75
Violet (mutant type)	14	7.25
Total	**193**	**100.00**

Figure 1. Flower colour variability (M3)

Reproductive performance and stability in the M4 generation

Evaluation of the M4 generation revealed that the majority of mutant lines retained reproductive ability. Out of 118 evaluated M4 mutant lines**,** 94 lines (79.66%) successfully produced pods and seeds (Table 2). This high proportion of fertile plants indicates that the induced mutations were largely compatible with normal reproductive development and that the mutant lines had reached a stable and usable stage. The maintenance of reproductive capacity in advanced generations is a key requirement for the practical utilization of induced mutants in breeding programs.

Table 2. Reproductive stability of mutant lines in the M4 generation

Reproductive status	Number of lines	Frequency (%)
Fertile (pods produced)	94	79.66
Non-fertile (no pods)	24	20.34
Total	**118**	**100.00**

Seed coat colour changes

Changes in seed coat colour were observed in several mutant lines, where the original yellow colour shifted to green or greenish shades (Figure 2). These changes were consistently expressed across generations, indicating stable inheritance. Seed coat colour is among the most frequently affected traits in mutation breeding of common bean and has been previously associated with gamma-induced genetic variation (Tomlekova, 2016; Sofkova-Bobcheva et al., 2021).

Figure 2. Seed coat colour changes

Alteration of growth habit

A notable modification of plant architecture was recorded among mutant lines. While the control accession displayed a determinate dwarf growth habit, several mutant plants developed a climbing growth form (Figure 3). Such changes represent major architectural mutations and are of agronomic relevance, as growth habit influences plant management and yield formation. Similar growth habit alterations have been documented in mutagenized populations of common bean and other legumes (Khursheed et al., 2019; Sofkova-Bobcheva et al., 2021).

Figure 3. Growth habit changes

Effect of irradiation dose

The 80 Gy treatment produced a higher number of viable and phenotypically stable mutant plants, whereas the 200 Gy dose resulted in stronger growth reduction and fewer stable mutants. These findings confirm that moderate gamma irradiation doses are more effective in generating useful phenotypic variation while maintaining plant viability (Kodhelaj and Ylli, 2017; Kumar et al., 2021).

CONCLUSIONS

Gamma irradiation successfully induced heritable phenotypic variability in the common bean accession “niska boranija”, marking the first application of induced mutagenesis in common bean breeding in Bosnia and Herzegovina. Stable morphological mutations, including flower colour change, seed coat colour variation, and altered growth habit, were confirmed through successive generations up to M4. Moderate irradiation (80 Gy) proved more suitable for generating useful and stable mutations while maintaining plant viability and reproductive performance, whereas the higher dose (200 Gy) resulted in increased physiological stress and reduced growth. The high proportion of fertile M4 mutant lines indicates that the developed material represents a valuable and stable resource. The newly established mutant lines constitute a promising foundation for further evaluation of agronomic performance, drought tolerance, and adaptability under local agroecological conditions, contributing to the diversification and improvement of common bean breeding programs in the region.

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