# DEVELOPMENT AND EARLY-GENERATION EVALUATION OF GAMMA-INDUCED VARIABILITY IN DRY BEAN (*PHASEOLUS VULGARIS* L.)

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## ABSTRACT

Gamma irradiation is an effective tool for generating genetic variability in grain legumes and expanding the breeding potential of elite cultivars. The aim of the present research was to develop and assess early-generation mutant populations of dry bean (*Phaseolus vulgaris* L.) obtained by using gamma irradiation and to identify superior plants for advancement in a mutation-breeding program. A total of 6 000 dry seeds were treated with three gamma ray doses (80, 120 and 160 Gy). In the M1 generation, emergence, survival and fertility showed a clear reduction, with the highest values recorded at 80 Gy and the lowest at 160 Gy. Despite these declines, sufficient number of fertile plants per each dose were attained to maintain adequate population sizes for further selection. High survival (>90%) and fertility were observed in the M2 generation, indicating the stabilization of viable mutant lines following the elimination of lethal mutations in M1. Substantial phenotypic variability was recorded in M2 for flowering time, maturity, plant architecture, plant height, pod number, seed number per pod and disease response. Several plants exhibited superior agronomic performance compared with the original breeding line. Selection was conducted on an individual plant basis using yield components, plant vigor, architecture, fertility and reduced disease symptoms as criteria, and selected plants were advanced to the M3 generation using the pedigree method. The results demonstrate that gamma irradiation effectively generated useful genetic variability in dry bean. Moderate doses provided an optimal balance between variability and plant survival, whereas higher doses increased phenotypic diversity but also caused greater biological damage. The selected M3 lines represent valuable genetic resources for further evaluation in replicated trials and for use in breeding programs aimed at improving yield, stress tolerance and disease resistance.

**Keywords:** gamma irradiation, bean mutants, early generations, phenotypic variability, M2 selection

## INTRODUCTION

Dry bean (*Phaseolus vulgaris* L.) is one of the most important grain legumes for direct human consumption worldwide due to its high protein content, dietary fiber, minerals and vitamins (Mullins and Arjmandi, 2021; Uebersax et al., 2022). It plays a key role in food security, particularly in developing countries where it represents a major source of plant protein. However,

the genetic base of many cultivated bean varieties is relatively narrow, which limits the potential for further improvement through conventional breeding (Cichy et al., 2015; Rendón-Anaya et al., 2017; Cortinovis et al., 2024).

Induced mutagenesis has been widely used as an effective approach for generating novel genetic variability in crops, including grain legumes (Ramandeep et al., 2018; Yali and Mitiku, 2022; Chen et al., 2023; Geras'kin, 2024). Unlike hybridization, which relies on existing allelic diversity, mutation breeding enables the creation of new alleles that may not be present in the available germplasm. This approach has contributed to the development of numerous improved crop varieties with enhanced yield, quality and stress tolerance. Recent advances emphasize the importance of mutant populations as valuable resources for breeding and functional genomics (Oladosu et al., 2016; Raina and Khan, 2023; Nanhapo et al., 2024; Rasmussen and Jain, 2024).

Gamma rays are among the most frequently used physical mutagens because of their high penetration capacity and ability to induce a wide spectrum of genetic changes (Ntsomboh Ntsefong et al., 2023). The effectiveness of gamma irradiation depends on the applied dose, which must balance mutagenic efficiency with plant survival. Excessively high doses cause severe physiological damage and reduce population size (Kumar et al., 2021), whereas moderate doses allow the recovery of viable mutants while still generating substantial variability. Recent studies have confirmed dose-dependent effects of gamma irradiation on germination, growth, pod formation and yield components in grain legumes (Supanjani et al., 2024).

Early mutant generations, particularly M2, are characterized by the expression of recessive mutations and therefore show a wide range of phenotypic variation. This segregation enables the identification of rare individuals with improved agronomic traits such as increased pod number, enhanced seed set, altered plant architecture or improved tolerance to biotic and abiotic stresses. The concept of mutant nurseries has recently been emphasized as a practical framework for evaluating large populations and selecting superior lines for advancement in breeding programs (Sofkova-Bobcheva et al., 2021; Rasmussen and Jain, 2024). In addition to agronomic traits, mutation breeding can also generate variation in disease response. Induced mutations may affect genes involved in plant defense mechanisms, leading to the identification of genotypes with improved tolerance to pathogens. This is particularly important for dry bean production, where diseases represent a major constraint to yield stability.

Environmental conditions can interact with mutagenic treatments and influence the expression of induced variation. Under field conditions, stress factors such as drought may enhance the expression of deleterious mutations, affect reproductive development and seed formation but also facilitate the identification of tolerant individuals, which has been reported in common bean (Ylli et al., 2022).

Despite the proven potential of mutation breeding, the development and evaluation of mutant populations in dry bean remain limited in many regions. The creation of well-characterized mutant populations is therefore an important step toward expanding the genetic base and supporting future breeding efforts.

The aim of this study was to develop gamma-induced mutant populations of dry bean and to evaluate their performance in early generations. Specifically, the objectives were to: (i) assess the effect of different gamma irradiation doses on emergence, survival and fertility in the M1 generation; (ii) evaluate survival, fertility and phenotypic variability in the M2 generation, and (iii) identify and select superior plants for advancement to the M3 generation as potential breeding material.

## MATERIAL AND METHODS

An advanced dry bean breeding line was used as the initial plant material. A total of 6 000 dry and healthy seeds were subjected to gamma irradiation at three doses: 80 Gy, 120 Gy and 160 Gy, with 2 000 seeds per treatment. All irradiated seeds were sown under open field conditions during the 2022 growing season to obtain the M1 generation. Emergence, survival and fertility (plants producing pods) were recorded for each treatment.

Seeds harvested from M1 plants were advanced to the M2 generation and 2 000 M2 seeds per irradiation dose, as well as seeds from the original breeding line were sown in the field in 2023. Plants were characterized for days to flowering, days to maturity, plant growth habit (determinate, semi-climbing and climbing), number of pods per plant, number of seeds per pod, plant height, yield per plant and disease response. Plants exhibiting superior agronomic performance compared with the original breeding line were selected and advanced to the M3 generation.

Emergence, survival and fertility were expressed as percentages. Descriptive comparisons among irradiation doses were used to evaluate dose-dependent effects. Phenotypic variation in the M2 generation was assessed qualitatively based on observed differences among plants. Selection of superior plants was conducted using visual evaluation of agronomic performance and yield components, following standard mutation-breeding procedures.

## RESULTS AND DISCUSSION

Gamma irradiation dose had a clear effect on plant establishment (Table 1). Emergence declined from 92.0% at 80 Gy to 82.6% at 160 Gy, while survival decreased from 87.6% to 76.5% of emerged plants. Fertility, expressed as the proportion of plants producing pods, also decreased with increasing irradiation dose, indicating increasing biological damage at higher irradiation levels. Nevertheless, a sufficient number of fertile plants were recovered in all treatments, enabling the advancement of mutant populations to the M2 generation.

The reduction in emergence and survival at higher doses is consistent with previous studies in common bean and other legumes, where increased irradiation levels caused cellular damage and impaired physiological processes, leading to reduced germination and plant viability (Supanjani et al., 2024). The decline in fertility reflects both mutagenic injury and environmental stress during the growing season, which resulted in flower abortion and the formation of seedless pods. Similar interactions between mutagenic treatments and environmental factors have been reported to reduce reproductive success in legumes (Ylli et al., 2022).

Despite these negative effects, the number of fertile plants remained adequate for maintaining population size and enabling subsequent selection. This balance between mutagenic effectiveness and biological damage is a key consideration in mutation breeding, as excessively high doses reduce population size, whereas moderate doses allow the recovery of a larger number of viable mutants (Oladosu et al., 2016; Rasmussen and Jain, 2024).

Table 1. Effect of gamma irradiation dose on emergence, survival and fertility in the M1 generation

| Dose (Gy) | Seeds sown | Emerged plants | Emergence (%) | Survived plants | Survival (% of emerged) | Plants with pods | Fertility (% of survived) |
|---|---|---|---|---|---|---|---|
| 80 | 2000 | 1840 | 92.0 | 1612 | 87.6 | 1385 | 85.9 |
| 120 | 2000 | 1765 | 88.3 | 1450 | 82.2 | 1176 | 81.1 |
| 160 | 2000 | 1652 | 82.6 | 1264 | 76.5 | 987 | 78.1 |

High levels of survival were observed in the M2 populations across all irradiation treatments (Table 2). Survival exceeded 90% in all cases, indicating that most viable M1-derived seeds produced stable plants in the subsequent generation. Fertility was also high and followed a similar trend, with slightly lower values recorded at the highest irradiation dose. The relatively high survival and fertility in the M2 generation reflect the elimination of lethal mutations during the M1 stage and the stabilization of viable mutant lines. This pattern is typical in mutation breeding, where early selection reduces the frequency of deleterious mutations and enables the recovery of agronomically stable genotypes (Sun et al., 2024; Chougale et al., 2025). The slightly lower values observed at 160 Gy suggest residual mutagenic effects on plant performance, although these effects were less pronounced than in the M1 generation.

From a breeding perspective, the high survival rate in the M2 generation allowed the evaluation of a large number of plants and increased the probability of identifying superior individuals, confirming that the applied irradiation doses were appropriate for generating useful mutant populations.

Table 2. Survival and fertility of $M_2$ plants derived from gamma-irradiated $M_1$ populations

| Dose (Gy) | $M_2$ plants emerged | $M_2$ plants survived | Survival (%) | $M_2$ plants with pods | Fertility (% of survived) |
|---|---|---|---|---|---|
| 80 | 1385 | 1312 | 94.7 | 1246 | 95.0 |
| 120 | 1176 | 1098 | 93.4 | 1025 | 93.3 |
| 160 | 987 | 892 | 90.4 | 812 | 91.0 |

Substantial phenotypic variation was observed in the M2 population across all irradiation treatments (Figure 1). Differences in flowering time and maturity ranged from early to late types, indicating that gamma irradiation affected developmental timing. Plant growth habit showed considerable diversity, including determinate, semi-climbing and climbing forms, reflecting mutations influencing plant architecture.

Plant height varied from dwarf to tall phenotypes, suggesting alterations in genes controlling vegetative growth. Wide variation was also recorded for pod number per plant, seed number per pod and yield per plant, with several individuals exhibiting superior reproductive performance compared with the original breeding line. Variation in disease response was observed under field conditions, where some plants showed reduced symptom expression, indicating the potential induction of alleles associated with improved tolerance.

Such broad phenotypic segregation is typical for M2 mutant populations and reflects the expression of recessive mutations following selfing of M1 plants (Oladosu et al., 2016). Comparable ranges of morphological and agronomic variation, including changes in plant stature, pod production, reproductive traits and yield components have been reported in mutant common bean populations, confirming that induced mutagenesis is an effective approach for expanding phenotypic diversity in breeding materials (Ylli et al., 2022; Supanjani et al., 2024).

Figure 1. Phenotypic variability observed in the M₂ generation of gamma-irradiated dry bean: variation in plant growth habit, plant height and architecture, flowering time and maturity, pod number and seed set, disease susceptibility and resistance

The presence of multiple phenotypic classes for each trait demonstrates the effectiveness of gamma irradiation in generating useful variability for selection and supports the use of mutant populations as reservoirs of novel genetic variation. Early-generation mutant populations are particularly valuable because they allow the identification of rare favorable phenotypes that can be fixed in later generations through pedigree selection (Oladosu et al., 2016; Rasmussen and Jain, 2024). The observed diversity in agronomic traits and disease response therefore provides a strong basis for the selection of superior plants and their advancement to the M3 generation for further evaluation.

Variation in disease response was observed among M2 plants under field conditions, allowing the identification of individuals with reduced symptom expression compared with the original breeding line. Plants showing minimal or no visible disease symptoms, combined with

normal growth and reproductive performance, were considered putatively tolerant and included among the selected genotypes for advancement to the M3 generation. In contrast, plants exhibiting severe symptoms, reduced vigor or premature senescence were discarded.

The occurrence of differential disease response in the M2 population indicates that gamma irradiation generated genetic variation affecting host–pathogen interactions. Similar observations have been reported in common bean mutant populations, where induced mutations resulted in altered reactions to fungal and bacterial pathogens and enabled the identification of lines with improved tolerance (Nanhapo et al., 2024). Mutation breeding has been widely used to develop disease-resistant cultivars in several crops, as induced mutations can modify resistance genes or regulatory pathways involved in plant defense (Oladosu et al., 2016; Sun et al., 2024). More recent studies emphasize the role of induced mutagenesis in generating novel alleles for biotic stress tolerance in grain legumes, supporting its use for developing climate-resilient cultivars (Rasmussen and Jain, 2024).

Selection for disease tolerance in early generations is particularly effective under natural field infection, as it allows the simultaneous evaluation of resistance and agronomic performance. The identification of M2 plants combining reduced disease symptoms with favorable yield components increases the probability of recovering stable resistant lines in later generations. This integrated selection strategy is consistent with mutation-breeding approaches that target both yield and biotic stress tolerance during early population evaluation (Raina and Khan, 2023; Rasmussen and Jain, 2024).

Selection in the M2 generation was conducted on an individual plant basis using visual evaluation of key agronomic traits. Plants were considered superior when they showed higher pod number per plant, increased seed number per pod, greater plant vigor and overall yield potential compared with the original breeding line. Additional selection criteria included favorable plant architecture (erect or semi-erect growth habit, bush type), uniform pod distribution, absence of visible disease symptoms and normal fertility. Plants exhibiting extreme, but agronomically undesirable phenotypes, such as severe dwarfism, sterility or poor seed set, were discarded. Selected plants were harvested individually and advanced to the M3 generation following the pedigree selection method. The identification of superior individual plants rather than shifts in population means is a fundamental objective of early-generation mutant evaluation. Early mutant populations represent heterogeneous genetic resources in which rare favorable alleles occur at low frequency, and effective selection therefore depends on the detection and advancement of outstanding individual plants (Oladosu et al., 2016; Supanjani et al., 2024). The advancement of selected plants to the M3 generation in this study is consistent with standard mutation-breeding strategies aimed at stabilizing desirable traits and enabling further evaluation in later generations.

The results from this study demonstrate that gamma irradiation effectively generated broad phenotypic variability in dry bean and enabled the identification of promising mutant lines. Moderate irradiation doses provided a favorable balance between variability and plant survival, whereas higher doses produced greater phenotypic diversity but also increased biological damage. Such responses are consistent with previous studies in common bean mutagenesis, which emphasize the importance of optimizing irradiation levels to maximize mutagenic efficiency while maintaining adequate population size for selection (Supanjani et al., 2024).

The selected M3 lines represent valuable genetic resources for further evaluation in replicated trials and for incorporation into breeding programs targeting yield improvement, stress tolerance and disease resistance. Their advancement to later generations will allow the stabilization of desirable traits and the confirmation of their agronomic performance under different

environmental conditions, which is essential for the effective utilization of induced variability in dry bean improvement programs.

## CONCLUSIONS

Gamma irradiation proved to be an effective method for inducing genetic variability in dry bean and for generating mutant populations suitable for early-generation selection. A clear dose-dependent reduction in emergence, survival and fertility was observed in the M1 generation, confirming the need to balance mutagenic effectiveness with biological damage when determining optimal irradiation levels. The high survival and fertility recorded in the M2 generation indicated the stabilization of viable mutant lines following the elimination of lethal mutations. Extensive phenotypic variation was observed for key agronomic traits, including plant architecture, reproductive characteristics and disease response, enabling the identification of superior individual plants. Selection based on yield components, plant vigor, architecture, fertility and reduced disease symptoms allowed the advancement of promising genotypes to the M3 generation.

Moderate irradiation doses provided the most favorable balance between population size and induced variability, while higher doses generated broader phenotypic diversity but reduced plant performance. The selected M3 lines constitute valuable genetic resources for further evaluation under replicated conditions and represent potential parental material for dry bean improvement programs targeting higher yield, stress tolerance and disease resistance.

Overall, the study confirms the usefulness of induced mutagenesis as a complementary breeding approach for expanding the genetic base of dry bean and supports its application in the development of improved cultivars adapted to diverse environmental conditions.

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