

## Review Article

# Role of Mutation Breeding by Gamma Rays to Improve Mung Bean. A Review

### Abstract:

Mungbean (*Vignaradiata L. Wilczek*) is one of the famous legume crops. Various biotic and abiotic factors affect the grain yield of mung beans. The yield can be increased by improving the genetic makeup and incorporating the resistance against environmental stresses. Common breeding methods are not useful in enhancing the production of mung bean because of low genetic variability. The production can be improved by improving the available genotypes through mutation or by using other advanced breeding methods. The present article will provide information about the use of mutation breeding in improving the grain yield of mung bean.

**Keywords:** Mutation breeding, Mung bean yield, *Vignaradiata L. Wilczek*

### INTRODUCTION

In Pakistan and many other nations throughout the globe, agriculture is a significant industry that supplies food for people and animals. Additionally, it aids the industries dependent on agriculture. In Pakistan, 70% of the population lives in rural regions, and they are all either directly or indirectly involved in the agricultural industry (Sangle and Lad 2020). ~~Mung bean~~ ~~It~~ is mostly grown in Asian nations and is also referred to as green gram. ~~One~~ ~~It is one~~ of the most significant legumes in several Asian nations, including China, India, and Pakistan. ~~It is and~~ a member of the *Fabaceae* family and *Papilionaceae* subfamily. It is the second most common crop in the ~~legume~~ ~~Fabaceae~~ family, after chickpeas (Jegadeesan and Punniyamoorthy 2023).

Mung bean is an inexpensive source of carbohydrates and proteins (24 ~~percent%~~) (38-50 ~~percent%~~). It also serves as a crop for animal feed. Dhal is made from split and dehusked seeds. When ~~it is~~ ~~the pods~~ ~~are~~ still green, it is used as a vegetable (Raina, Laskar et al. 2023). It is prioritized over other pulses since ~~it is simple to absorb~~. Because of its capacity to withstand dryness, it ~~is seeded~~ ~~can be grown~~ in a range of ~~environmental~~ conditions and may thus be planted in both desert and irrigated locations. Mung bean production is controlled by environmental and genetic variables (Huppertz, Kachhap, et al. 2023). By enhancing the genetic makeup and introducing tolerance to environmental challenges, the yield ~~may~~ ~~can be~~ ~~raised~~ ~~increased~~ (Kharkwal 2023).

Conventional breeding methods are inefficient for boosting production in mungbeans because of their low genetic diversity. The production may be boosted by using more cutting-edge breeding methods or by employing mutation to improve the existing genotypes (Mir, Maria et al. 2020). Mutation breeding is one of the ~~first~~ ~~methods of breeding~~. ~~It is used in various fields, including~~ ~~biotechnology~~ ~~Biotechnology~~, cytogenetics, and molecular biology ~~technologies are applied in mutation breeding~~. It is an effective tool for ~~raising~~ ~~increasing~~ agricultural production. Mutations may be ~~brought on~~ ~~induced~~ by gamma rays and other kinds of physical and chemical mutagens. Gamma rays affect plant development by altering the genetic, physiological, biochemical, and morphological properties of the cells (Pathak, Panchariya et al.

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2023). According to Khatri et al. 2005's study, gamma rays with EMS may generate high-yielding unique types(KHURSHEED, WANI et al. 2021).

Inducing mutations is one of the key methods for boosting genetic variety and is usefully used to develop agricultural genotypes with enhanced yields. The efficacy and efficiency of the mutagens affect mutation breeding. Efficiency is connected to unfavorable alterations including sterility, damage, and fatality, as well as to mutation per unit dosage of mutagens(Masry, Fayad et al. 2021).The choice of effective and efficient mutagens determines the desired mutation (Pramanik, Debnath et al. 2023). If the areas not affected by the first mutagen are exposed to the action of the second, the two mutagens working sequentially one after the other may create more than an additive impact(Kharkwal 2023).

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The mutation is the abrupt alteration of the gene sequence. Both the ~~plant's~~ seed and ~~its~~ vegetative component are susceptible to ~~inducing~~ induced mutations(Datta 2023). ~~The investigation of the structure and function of the genes is prompted by vaccine mutation.~~ Induced mutations have been employed to improve cultivars of cereals, fruits, and other crops by enhancing their natural genetic sources (Anuragi, Yadav et al. 2022). ~~Mutation has a practical role in plant breeding.~~ Around 2252 mutant types have been created over the last seven decades(Badigannavar, Mondal et al. 2022).According to (Ahir, Kumar et al. 2023), the bulk of mutant types were created using gamma rays. They noted that gamma rays may alter an organism's genetic makeup, disrupt gene linkages, and develop a variety of novel features with the potential to enhance agricultural plants (Atteh and Adeyeye 2022).The present ~~kinds varieties~~are variable because of mutations, and early maturing lines may be created that are useful in the summer. Mutation breeding is the finest technique for creating superior crops (Magdy, Fahmy et al. 2020).

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Analysis of the induced mutation may be used to determine the heritability of critical qualities including productivity, disease and pest resistance, and quality. ~~Pulses' P~~oor productivityof pulses may be due to their vulnerability to pathogenic microbes, asynchronous pod maturation, flower shedding, freshly formed pods, and indeterminate to lengthy periods of growth, which results in low seed production per plant(Ma, Mohd Raffi et al. 2023). The plant can also fix nitrogen in the soil biologically, preserving the soil's fertility.Different statistical methods have been used by various researchers to assess the grain production of various crops(Muhammad, Rafii et al. 2021).

**Comment [Ma8]:** Discuss the importance of legumes and Mung bean before discussing its drawbacks

MutationsIn fact, mutations have played such a significant role in evolution that they were formerly thought to be the primary driver of the emergence of new species. It is now generally known that mutation, when combined with hybridization, is the ultimate source of new variety. This new genetic diversity is crucial for the development and improvement of food plants. Both naturally occurring and purposefully caused mutations are possible(Yasmin and Arulbalachandran 2022). Mutation breeding, which has been widely utilized to create novel crop cultivars and modify plant attributes, uses induced mutants in breeding programs to create better kinds. More than 2500 kinds have been made available worldwide over the course of the last eight decades, either as direct mutants or via their offspring(Balvoa-Caguana, Heredia-Moyano et al. 2021). Enhancing the germplasm has been based on gene mutations that enhance well-adapted current cultivars' qualities or create new cultivars by exploiting mutant traits (Balvoa-Caguana, Heredia-Moyano et al. 2021). The prime strategy in mutation-based breeding has been to upgrade the well-adapted plant varieties by altering one or two major traits which limit their productivity or enhance their quality value.

**Comment [Ma9]:** After discussing about pulses don't come back to discuss all the crops. Make an attractive flow in organizing the information gathered.

Mutations have had such a significant impact on evolution that they were formerly thought to be the primary driver of the emergence of new species. It is now generally acknowledged that new kinds are produced by a combination of hybridization and mutation(Yali and Mitiku 2022). This new genetic variation is crucial for the development and growth of food plants. Mutations can occur both accidentally

and on purpose. The process of mutation breeding, which has been widely utilised to create utilized drop cultivars and change plant features, uses induced mutants in breeding programs to produce programs kinds. More than 2500 types have been made available on a worldwide scale during the last eight decades, either as direct mutants or via their offspring (Stebbins 1974). Gene mutations have been utilized to create novel cultivars or to enhance the traits of well-adapted cultivars that are currently in use. By modifying one or two crucial traits that either decrease or raise a plant type's productivity, mutation-based breeding has mostly sought to enhance well-adapted plant kinds (Khan, Rafii et al. 2021).

**Comment [Ma10]:** Repetition of the previous paragraph. In a review, do not copy and paste everything in publications. Try to understand the facts and make them in a sequence to have a sense.

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### Types of Mutations

~~The following two classifications may be used to classify~~ The agents available for inducing mutations, or mutagens can be classified in to two groups—X-rays, gamma rays, UV radiation,  $\alpha$ -particles, neutrons, and particles from accelerations are examples of physical phenomena. Base analogs, antibiotics, alkylating agents, acridines, azides, hydroxylamine, and nitrous acid are among the chemical substances (Joshi, Ghimire et al. 2023).

Despite a variety of mutagenic agents being readily available, it is still challenging to coordinate the induction of mutation with intended character expression. Ionizing radiations, such as X-rays and gamma rays, are often selected because of their ease of use, excellent penetration, repeatability, high mutation frequency, and few disposal issues. The UV rays can only penetrate a small amount of tissue, which limits their application to pollen grains (Jenkins, Juba et al. 2023). However, UV radiation works quite well to irradiate protoplasts and cells of grown plants. The radiation dosage depends on the explosive's length and radiation intensity. It is measured in terms of the number of ionizations that take place and is given in Roentgen (R) units. Irradiation dosage is often stated in terms of kR or Gray (Gy) in mutant breeding studies, where 1 Gy = 100 rad and 1 kR = 10 Gy. Radiation absorbed dosage is measured in units of rad, where 1 rad equals 100 erg/g or 10<sup>-2</sup> joules/kg and is represented as rad per second, minute, or hour (Chandora and Rana 2023).

The most often utilized chemical mutagens include ethyleneimine (EI), diethyl sulfate (DES), methyl nitroso urea (MNH), ethyl nitroso urea (ENH), and ethyl methane sulphonate (EMS), which are all members of a particular family of alkylating agents. All of these substances interact with DNA by alkylating the purine and pyrimidine bases as well as the phosphate groups. A chemical mutagen's dosage is primarily influenced by concentration, treatment time, and treatment temperature. Pre-soaking, solution pH, metallic ions, carrier agents, post-washing, post-drying, and seed storage are modifiable parameters. EMS dosages for causing morphological mutations range from 0.01 to 0.8 percent. From species to species, there are very few variations in the dosage to be employed in the therapy (Singer, Laurie et al. 2021).

**Comment [Ma13]:** Explain why gamma irradiation is important /advantageous over other mutagens.

According to Malik et al. (1998), 250 Gy was determined to be the 50% fatal dosage for inducing chlorophyll and morphological changes in lentils. The effective dose of gamma rays varied from 214 to 218 Gy (LD 50) (Krug, BM Drummond et al. 2023). On the other hand, discovered that the lowest chlorophyll mutation frequency occurred at 200 Gy and the greatest at 600 Gy. The largest number of mutations are produced with the least amount of risk at the mutagen's optimal dosage. A preliminary therapy may be used to identify an ideal dosage. Overdosing on mutagens will result in a large number of plant deaths, whilst underdosing will result in low mutation rates. Lower frequencies, however, could have the benefit of inducing fewer undesired background mutations in addition to the desired mutation (Dolatabadian and Fernando 2022). The outcome of induced mutations has been discovered to be influenced by several variables, including native plant species, target tissue, water content in the target tissue, temperature, oxygen level, and dosage rate. The water content of the seed has a significant impact on the frequency of mutations. Irradiating seeds that contain 12–14% water often result in a greater mutation frequency. In chemical mutagenesis, pre-soaking seeds in water for 8–12 hours and post-treatment drying are crucial (Koundinya, Das et al. 2023).

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### Treatment with Mutagens and Mutant Selection

#### Choice of Material to be treated

To accomplish clearly stated goals via mutant breeding, the selection of parent material is essential. Programs for breeding desirable mutations should be carefully thought out, well organized, and big enough to pick out the low frequencies that are most likely to occur. Improvement of regionally adapted variants or the creation of novel alleles not present in germplasm collections may be the goals of mutation breeding (Chen, Wong et al. 2022). While a locally adapted variety must be employed when the goal is to introduce a new variety via mutation breeding, any plant introduction may be used to develop new germplasm for use in cross-breeding. The variety chosen for mutagenesis must be among the finest recently released kinds. Two or more kinds must be used for mutagenesis because various genotypes respond to mutagens differently. Selecting a high-producing variety with good adaptation is usually useful for enhancing one or two particular features (Dave, Raad et al. 2022). All plant components may be altered in some way, particularly when using a certain kind of mutagen. Although treating seeds is more usual, crops that are grown vegetatively may also benefit from treating entire plants, dormant cuttings, bulbs, tubers, and corns. Before treatment, soaking seeds in water produces favorable outcomes. The most often treated material is seed because it can withstand physical conditions that are typically only tolerated by inanimate objects like molecules. When treating seeds, the LD 50 dose—enough to prevent germination by around 50%—is often employed to achieve positive outcomes. A mutagen's dosage and pace, or length of administration, vary depending on the type of plant and should be established via testing (Ning, Li et al. 2023).

#### **Monogenic features are chosen in mutants**

The kind of population created by mutagenesis treatment relies on the agricultural plant's reproductive strategy and the genetics of the intended improvement in character. The following plan may be employed in a mutant breeding program to enhance a variety for a characteristic controlled by main genes (Quarleri, Galvan et al. 2022):

(i) suitable mutagen is used to treat 500–600 seedlings. The M1 generation consists of plants that are grown from treated seeds. The M1 is heavily planted, and each plant's seeds are collected individually. It is important to carefully watch the M1 plants to detect any dominant alterations (Whitfield and Huse 2022).

(ii) In plant-to-progeny rows, the M2 generation is produced from seeds collected from M1 single plants. Plants are collected individually from progeny rows thought to contain mutant alleles. M2 exhibits recessive mutations. The size of the M2 generation should be sufficient to meet the targeted goals of the mutant breeding program (Reichert, Morgan et al. 2023).

(iii) To produce the M3 generation, the chosen M2 plants are cultivated in progeny rows. While the heterogeneous progenies are subsequently exposed to the selection of mutant plants, if any, which can be cultivated to develop M4 homogeneous progenies, the homogeneous mutant progenies are collected as bulks in the M3 generation (Ning, Li et al. 2023).

(iv) The uniform mutant progenies from the M4 generation are ideally further assessed in repeated trials. If there are any segregating descendants, they must be discarded.

(v) In M5, the better mutant offspring are assessed in an initial yield experiment coupled with an appropriate check. Before being introduced as a new variety, the potential stable mutant progenies/lines undergo further testing over the course of two to three years in many locations. You may save the low-yielding mutant lines and utilize them in a hybridization procedure to add variety (Ning, Li et al. 2023).

#### **Mutation breeding for polygenic traits**

Polygenic features experience genetic variation as a result of induced mutagenesis. Sometimes irradiation causes the mean of the M2 population to fall, but some M3 families are superior to the control or F2 populations. This variance is often up to 50% of that created in the F2 generation. Therefore, both positive and negative mutations are produced. Positive mutations provide opportunities for selection-based enhancement of polygenic features. M1 and M2 are grown in the same manner as monogenic characteristics for polygenic traits (Ningwal, Tripathi et al. 2023). The robust plants in M2 are chosen and harvested individually to produce distinct plant offspring in M3. In the M3 generation, a few homogenous rows are harvested as bulks while inferior progeny rows are discarded. The discovery of mutants with changed quantitative features, such as partial or horizontal disease resistance, is aided by careful

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inspection of a large number (1000) of M3 progeny rows(Udayasri, Reddy et al. 2022). Bulk seeds from M3 rows that are uniform may be evaluated in M4 yield trials with a proper check. The superior offspring are next put to the test in repeated, multi-location yield experiments with an appropriate check. Again, M4 may reject the segregating progenies. Outstanding homogenous progenies may be offered as new kinds after two to three years of testing at several locations(Gulwane, Deore et al. 2022).

### Benefits of breeding for mutations

A crop species may be made very variable by mutation breeding, which can then be exploited to improve the germplasm and generate new cultivars. As opposed to hybridization, mutant breeding has a significant time benefit when creating new varieties. In general, it takes 11–12 years to generate a new variety via hybridization, but mutation breeding only needs 8–9 years(Tengse, Sarode et al. 2022).

Altering alleles at both known and recently undiscovered loci, as well as linkage groups, allows mutations to produce both qualitative and quantitative diversity (Pravallika and Lavanya 2022). Leguminosae exhibit variety that is limited to a level that is present in the parents used in hybridization, although new variability may be created by mutations for many desirable features (Kushwah, Jain et al. 2022). If the gene is present in the treated plant, mutagenesis may produce any form of variant seen in any plant family's germplasm(Kushwah, Bhatia et al. 2022).

### Difficulties

For various plant species, the mutation frequency varies, and even within a species, there are variations in the genotypic responses to various mutagens and their various treatments (Singh, Nath et al. 2023). The prevalence of advantageous mutations is very low, at around 0.1 percent. The strategies utilized to manage various mutant generations, the efficacy of screening approaches, and the size of the population developed in M1 and subsequent generations all have a role in how successful mutation breeding is. The bigger M2 population should be cultivated and rigorously examined at various phases of growth and development to extract attractive mutants to achieve a greater success rate. Large-scale population screening is more time-consuming, labor-intensive, and costly in terms of manpower and resources(SRIDHARA, NANDAGAVI et al. 2022). While some mutations are undesirable since they cause chromosomal abnormalities and deletions, others have pleiotropic effects caused by related gene(s). Such mutants sometimes need backcrossing to parents or modified types, which is time-consuming and labor-intensive once again. Furthermore, it is difficult to break the unfavorable connection between a gene of interest and bad genes, which limits the application of mutation breeding for crop improvement regularly(Arriagada, Cacciuttolo, et al. 2022).

### Mutation in MungBean

In Mungbeans, since there is little variation in the germplasm, mutation breeding is highly effective. ~~Mutagens are substances that produce variation.~~ In the past, physical and chemical mutagens were employed to examine the mutagenic impact onmung bean; as a result, the issue of varietal development also arose(Verma, Singh et al. 2023).

Nearly 34 types of diverse pulse crops have been established for commercial production thanks to mutation breeding, while 97 variants have been created on a global scale(Jamil, Ilyas, et al. 2022). Gamma rays have allowed for the development of the greatest variety (Table 1). These days, breeding programs routinely utilize induced mutants to impart the desired characteristic. Below are some noteworthy developments achieved in the pulses enhancement program via mutation(Yasmin, Arulbalachandran et al. 2022).

~~In South and South East Asian nations, mung bean, often known as green gram, is a significant pulse crop.~~ The area regarded as its center of origin includes the Indian subcontinent. The nation has seen the emergence of several better kinds, some of which were created via mutation (Table 2). Gamma irradiation is said to boost the protein content and yield in mungbeans, according to mutation research (Yasmin, Arulbalachandran et al. 2022). Mutations caused by mutagens such as gamma rays, EMS, and epichlorohydrin produce plants with noticeably more pods, seeds, 100-

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seed weights, and seeds per plant (Sarma, Dhole et al. 2022). Additionally, gamma rays can produce semi-dwarf plants, mutant pods, and leaf varieties (Sarma, Dhole et al. 2022). In mung bean, EMS over 0.03 M concentration exhibits excellent efficacy and efficiency. (Khan, ABIDEEN et al. 2023) found a linear association between rising EMS dosage and a decrease in biological parameters such as germination, height, survival, maturity, and pollen fertility. According to (Vanniarajan and Chandrakala 2022), when it comes to causing damage to seedlings, sterilizing pollen, and inhibiting germination, EMS has the greatest level of mutagenic effectiveness, followed by MMS and SA. While the EMS indicates a rising tendency with dosage on fertile branches, pods per plant, and plant height, it consistently shows a reduction in plant height, days to blooming, and days to maturity as concentration increases (Zhao, Meng et al. 2022). High potential improvements in terms of plant height, days to blossom, and maturity may be obtained by selection in the M2 generation. (Lal, Lavanya et al. 2022) used EMS to produce branchless and multifoliate mutants of mungbean. The days to blooming, days to maturity, and plant height mean values likewise reveal a negative shift as a result of the EMS and SA mutagenic impact (Shahzadi, Nawaz et al. 2023). The effectiveness and impact of mutagens are greatly influenced by genotype; the consequences of mutation may also vary significantly depending on the kind and amount of mutagens utilized.

Name of Variety	Year of Release	Center of Research	Parent Variety	Mutagenic Agent	Area Recommended For Cultivation	Varietal Characteristics
CO-4	1982	TNAU, Coimbatore	CO-1	20kR Gamma rays		High yielding, 85 days maturity and drought tolerant
PantM 2	1982	GBPAUA & T. Pantnagar	ML-26	10kR Gamma rays		High yielding, resistant to YMV
TAP-7	1982	BARC, Mumbai and PVK Akola	S-8	Gamma rays		Early by 5-7 days and 23% higher yield than Kopergaon
MUM2	1992		K851	--	North East Plain Zone	Early, dwarf type, resistant to MYMV
BM4	1992		T44	--	Central Zone	Bushy erect type, tolerant to MYMV and powdery mildew
TARM-2	1994	BARC, Mumbai	TPM-1 x RUM-5	--	Maharashtra	High yielding, resistant to powdery mildew
LGG407	1995		PantM 2	--	Andhra	tolerant to

Table 1 : Details of Mung bean varieties developed through mutation breeding in ( your country)

The majority of mutant types are discovered to be YMV resistant. In 1982, three varieties—'CO-4' from TNAU in Tamilnadu, 'Pant Moong-2' from Pantnagar, and 'TAP-7' from BARC in Mumbai—were made available. Following that, in 1992, the introduction of early maturing cultivars resistant to the yellow mosaic virus (YMV) and their appropriateness for various cropping techniques led to a rise in both area and output in several states, including Bihar, Gujarat, Maharashtra, Rajasthan, and Punjab. Several mutant cultivars, such as "MUM 2," "BM 4," "LGG 407," "LGG 450," "CO 4," "Dhauri" (TT9E), "Pant Mung-1," and "Tap 7" are among these varieties (Ahir, Kumar et al. 2023). The majority of mutant cultivars have early maturation, large yields, and YMV tolerance or resistance.

Nine mutant mungbean cultivars that mature early and uniformly, are low in height and contain huge seeds have been introduced in Pakistan. NIAB Mung 92 and NIAB Mung 98 were authorized for cultivation in the Punjab Province in 1996 and 1998, respectively. Both cultivars have good yields and are resistant to Cercospora leaf spot and the YMV virus. The summer mungbean cultivar SML 668 was created by selecting for YMV resistance and synchronous maturation from a mutant line called "NM 94." (Ahmad, Jan et al. 2023). In the states of Punjab, Haryana, Himachal Pradesh, Rajasthan, and Bihar, SML 668 is particularly well-liked.

### Prospects

Pulse genomic research is accelerating in the biotechnology era that has replaced the cereal age. We will need fully developed genomic information and populated genetic maps in the future. For the study of gene regulation and expression, mutants are necessary. Even though some plant genomes have been sequenced, assigning functions to many DNA sequences requires inducing mutation using gamma rays, fast neutrons, or chemical mutagens (Ahloowalia and Maluszynski, 2001). The mutants that researchers often discard because they are unfit for breeding are now crucial tools in the genomic study. In pooled samples acquired from sizable mutant populations employing innovative mutation detection technology, damage to the DNA of a specific gene sequence is now feasible. This method, sometimes referred to as "targets including local lesions in genomes" or "TILLING" (McCallum et al., 2000a), is becoming more and more well-known these days. Through the use of "denaturing high-performance liquid chromatography," also known as "DHPLC," this innovative reverse genetic method combines a high frequency of point mutations brought on by unique mutation procedures. In this method, high-density point mutations are needed, which is why extremely effective chemical mutagens and ionizing radiation are often utilized to create mutant generations. Although McCallum et al. (2000b) initially used Arabidopsis to show this strategy, other plant species have since used it as well. TILLING may be effectively utilized if the sequences of the targeted gene are known and the mechanism for detecting single nucleotide alterations is available. In a population with variation induced by any mechanism, choosing the desired genotype is difficult. Molecular probes now make it feasible to choose the mutations that were found. In the future, sequencing of the altered genes and the creation of molecular markers will garner greater attention to enhance pulse. Using mutation breeding and markers-aided selection, cultivars with higher efficiency in terms of absorption of micronutrients, tolerance to abiotic conditions like drought, cold, and salt, and resistance to biotic stresses like disease and insect pests may be created.

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