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Review article

# Status of mutation breeding in creating novel varieties of crops: A Review

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## SUMMARY

Mutation breeding has gained widespread popularity among breeders for developing elite breeding lines. Mutation serves as the fundamental source of all genetic variation in organisms, including plants. Many mutant varieties have been created through mutation breeding, which can occur spontaneously or be induced. Spontaneous mutations happen naturally at a low frequency, while induced mutations are intentionally generated by humans using agents known as mutagens. Mutagens include chemical agents like ethylmethanesulphonate (EMS), sodium azide, and nitrous acid, as well as physical agents such as X-rays, gamma rays, and fast neutron irradiation. Biological mutagens, such as DNA transposons, retrotransposons, and insertion sequences, also play a role in induced mutagenesis. This process has significantly contributed to the development of novel varieties with enhanced and desirable genetic traits. According to the Food and Agriculture Organization (FAO), over 3,220 mutant varieties of crop plants have been developed, with most being created using physical mutagens like gamma rays and X-rays. Among these mutant varieties, nearly 80% are seed propagated. Nearly 50% of the mutant varieties developed are cereals and legumes, including rice, wheat, maize, barley, cowpea, groundnut, and soybean. Mutation breeding has been instrumental in addressing the growing demand for food and proper nutrition and has also contributed to the economic growth of countries worldwide.

**Keywords**: Mutation breeding, Novel varieties, Biological Mutagens, Physical agents, EMS, Crop improvements

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## INTRODUCTION

Globally, the human population is continually rising and is projected to reach nine billion by 2050, potentially leading to significant food shortages [1]. To meet the escalating demand for food and ensure adequate nutrition, urgent advancements in food production are necessary [2]. A mutation refers to a sudden, inheritable alteration in the DNA of a living cell, not resulting from genetic segregation or recombination [3]; [4]. Mutation breeding involves the intentional use of mutations in plant breeding, which can be considered as human-directed evolution of crop plants, with genetic variation as its foundation [5]. Mutagenesis, the process of inducing sudden inheritable changes in an organism's genetic makeup, can be achieved through chemical, physical, or biological agents [6].

Mutation breeding has gained popularity among breeders for developing superior breeding lines and cultivated varieties while maintaining desirable quality traits [4]. It is most commonly applied in asexually propagated and self-pollinated crops to create variations that can improve morphological and physiological traits, disease resistance, and quantitative characteristics such as yield. Mutation is the primary source of all genetic variations present in organisms, including plants [7]. Plant mutation breeding is widely recognized as a legitimate technique for increasing genetic diversity in plants, particularly in commercially important crops. It is considered more efficient and less time-consuming. With advances in science, mutation breeding techniques have significantly evolved over recent decades. Most mutation breeding methods rely on mutagenic agents, which induce mutations in the plant's genetic material [8]. The variations produced by mutations provide the raw material for natural selection and act as a driving force in evolution, making it a powerful tool for creating new plant varieties. The genetic enhancement of crops is a critical element in addressing global food security and nutrition challenges [9]. It is estimated that food production needs to at least double by 2050 to meet the demands of the ever-growing population [10]

### History of Mutation Breeding

Mutation refers to a sudden and inheritable alteration in an organism's phenotype. The term was first introduced by Dutch botanist Hugo De Vries (1848–1935) [11]. De Vries initially defined mutations as abrupt, inheritable changes in genetic material that were not caused by recombination or segregation [12]. He used the term "sudden" to distinguish these changes from the gradual alterations that can occur through recombination. However, with advances in modern techniques, it became evident that mutations, especially at the genetic level, can cause subtle phenotypic changes that may not be immediately apparent. Consequently, the word "sudden" was eventually removed from the definition [13]. Mutations are crucial in evolution, as inherited changes in genetic material can lead to novel phenotypic traits [14]. The concept of mutation breeding was first introduced by Freisleben and Lein in 1944, describing the intentional induction and development of mutant lines for crop improvement. The practice of improving crops through induced mutations began around ninety years ago, following the discovery of the mutagenic effects of X-rays on Drosophila by Muller in 1927 [15]; [16] and on barley and maize by Stadler in 1928 [17].

In the 1950s and 1960s, countries like the United States and Japan began using mutation breeding to enhance crops. To date, over 3,220 mutant varieties of crop plants have been developed [18], with most being created using physical mutagens like gamma rays and X-rays. Among these mutant varieties, nearly 80% are seed propagated. According to the Food and Agriculture Organization (FAO), nearly half of these mutant varieties are cereals cultivated globally [19].

## Mutation Breeding

Mutation breeding is defined as the intentional induction and development of mutant lines to improve crops [7]. Induced mutations serve as an alternative source of genetic variation for plant improvement programs and as an alternative to hybridization and recombination in plant breeding. A mutation is a heritable alteration in an organism's genetic characteristic, creating new gene variants (alleles). This natural process is the primary source of all genetic variation in organisms, including plants [20].

## Process of Mutation Breeding

Mutation breeding involves three main steps:

1. **Mutation Induction**: This step involves exposing plant material to biological, chemical, or physical mutagens to enhance desirable or targeted plant characteristics [21].
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2. **Mutant Screening**: In this process, individuals from a large, mutated population are selected based on specific criteria. For instance, M2 plants that flower earlier than their wild-type parent(s) is screened as potential early flowering mutants, and plants without disease symptoms are screened as potential disease-resistant mutants. However, since traits like flowering depend on both genetic and environmental factors, these plants are considered "putative mutants," meaning they are not necessarily "true mutants." This situation applies to many traits, including disease resistance, where the absence of infection may be due to the absence of the pathogen [22].

3. **Mutant Verification**: This step involves re-evaluating the putative mutants under controlled and replicated conditions, using larger sample sizes (usually the progenies of selected putative individuals, e.g., M3 lines of selected M2 plants). Many putative mutants, especially for quantitative traits like growth duration, yield, quality, and disease resistance, may turn out to be false mutants [22]. The success of mutation breeding depends on three factors: the efficiency of mutagenesis, the starting plant material, and the mutant screening process [23].

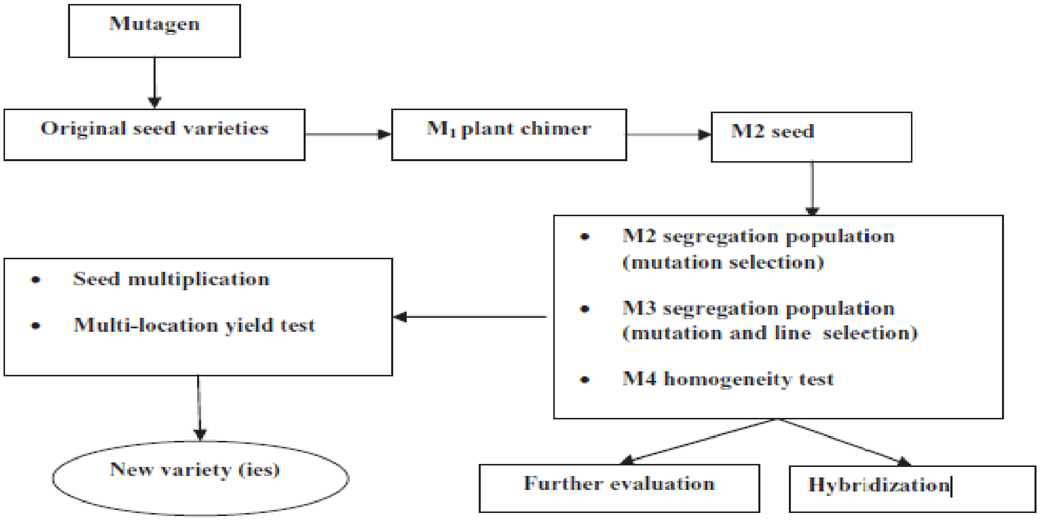

**Figure 2.1: Process of Mutation Breeding**

Source: [24]

### Mutagenesis

Mutagenesis is a process where an organism's genetic information is altered due to exposure to mutagens, leading to mutations [25]. Mutations can happen naturally at a very low rate or be induced experimentally through physical and chemical mutagens [26]. The integration of advanced molecular biology techniques and in vitro culture methods with mutagenesis has significantly enhanced crop improvement and breeding programs, especially in the context of global climate change [11]. Mutagenesis is commonly performed using three major approaches: biological agents such as transposons, retrotransposons, and T-DNA; physical agents like ionizing radiation; and chemical agents, including alkylating agents and azides [27].

### Types of Mutagenesis

### Spontaneous Mutation

Spontaneous mutations in crop plants occur naturally during adaptation and evolution at a very low frequency, approximately 10-5 to 10-8. This low frequency is generally insufficient to create the genetic variation needed for significant crop improvement [28]. Due to their low frequency, spontaneous mutations are typically useful only in limited cases, particularly in vegetatively propagated ornamental or fruit crops. These mutations arise from errors in natural biological processes without any human intervention [29]. Spontaneous mutations often lead to the discovery of new alleles of previously unknown genes, and these alleles are identified by their phenotypic effects. Additionally, spontaneous mutations and deletions can help identify critical functional regions within a gene.

## Induced Mutation

Induced mutations occur at a much higher frequency than spontaneous mutations. The induction of mutations generates genetic variability without requiring prior genetic improvement. However, there is no assurance that a 'mutant plant' will have only one genetic mutation; in fact, multiple mutations may be induced, affecting thousands of genes [29]. Induced mutations, caused by treatment with physical or chemical mutagens, are highly effective in creating genetic variability for various economically important traits in crop plants. Induced mutations have been successfully used to increase genetic variability in crops such as barley, oats, and wheat. The agents used to induce mutations are known as mutagens. Some genes in an organism can promote the mutation of neighbouring genes on the chromosome. Induced mutagenesis is recognized as a safe, robust, and cost-effective technique for plant breeding [30]. Mutations can be induced using physical mutagens (e.g., gamma radiation, high and low energy beams) or chemical mutagens (e.g., ethyl methanesulfonate, EMS), applied to both seed and vegetatively propagated crops. The mechanism behind mutation induction involves the breaking of nuclear DNA by the mutagen, followed by the occurrence of new mutations during the DNA repair process, which are heritable. Mutations can also affect cytoplasmic organelles and lead to chlorophyll mutations, as well as chromosomal or genomic mutations. These mutations provide plant breeders with useful traits such as resistance to abiotic and biotic stresses [31].

## Mutagenic Agents

Mutagens, the agents that induce mutations, are generally classified into three broad categories: chemical mutagens, physical mutagens, and biological mutagens.

## Physical Mutagen

Physical mutagens include all forms of nuclear radiation and radioactivity, such as ultraviolet light (a non-ionizing radiation), various types of ionizing radiation (e.g., X-rays, gamma rays, alpha particles, beta particles), and subatomic particles like protons and neutrons [32]. Over the past 80 years, physical mutagens—especially ionizing radiations—have been widely used to induce genetic changes, with more than 70% of mutant varieties developed through physical mutagenesis. Radiation refers to energy travelling in the form of waves or particles over a distance. High-energy levels within the electromagnetic (EM) spectrum can dislodge electrons from the atomic nuclei they impact, thereby ionizing the atoms. Ionizing radiation includes cosmic rays, gamma rays, and X-rays [15].

X-rays were the first physical mutagen used to induce mutations. Since their discovery, various subatomic particles, such as neutrons, protons, beta particles, and alpha particles, have been generated using nuclear reactors. Gamma radiation, particularly from radioactive cobalt-60 (60Co), is widely used due to its high penetrating power, despite being hazardous. Gamma radiation can irradiate whole plants and delicate materials, such as pollen grains. Many mutants have been developed using gamma radiation, which primarily causes DNA double-strand breaks that lead to mutagenic effects.

**Table 1: Example of commonly used physical mutagen**

| Mutagen | Source | Characteristics | Hazard |
|---|---|---|---|
| X-rays | X-ray machine | Electromagnetic waves; can penetrate tissues | Dangerous, penetrating |
| Gamma rays | Radioisotopes and nuclear reaction | Strong electromagnetic waves; deep tissue penetration | Very dangerous, highly penetrative |
| Neutrons | Nuclear reactors or accelerators | Uncharged particles; penetrate tissues deeply | Very hazardous |
| Beta particles | Radioactive isotopes or accelerators | Electrons with shallow penetration | Potentially dangerous |
| Alpha particles | Radioisotopes | Helium nuclei; very shallow penetration | Very dangerous |
| Protons | Nuclear reactors or accelerators | Hydrogen nuclei; moderate tissue penetration | Very dangerous |
| Ion beam | Particle accelerators | High-speed ions with strong energy impactspeed. | Dangerous |

Source: [33]

### 2.2.2 Chemical Mutagen

Chemical mutagens are defined as those compounds that increase the frequency of some types of mutation. Chemical mutagens were found to be highly effective in inducing true gene mutations and the specificity of action could be investigated through analysis of their reaction with different DNA bases. Chemical mutagenesis has become a widely adopted approach because it does not require special facilities, and the resulting mutations are primarily SNPs. Ethyl methane sulfonate (EMS) is currently the most commonly used chemical mutagen, but methylnitrosourea, sodium azide,di-ethyl sulfate and diepoxybutane have also proven effective [34]. The modes of action of a chemical can influence the mutations induced. For example, EMS selectively alkylates guanine bases, leading primarily to GC to AT transitions [35].

### Biological Mutagens:

DNA transposons, retrotransposons, and insertion sequence (IS) elements are recognized as biological mutagens, commonly referred to as transposable elements in molecular biology. These elements are categorized into two primary classes, Class I and Class II, based on their transposition mechanisms [36].

Research on biological plant mutagens is currently at a relatively early stage. However, as science and technology advance, research in this area is expected to progress. Emerging biotechnological methods and molecular biology techniques, such as transposon technology, will drive the development of biological mutagens. Technologies like the CRISPR-Cas9 system hold significant potential for plant mutation breeding, given their ability to precisely modify target DNA [37].

**Table 2: Example of commonly used chemical mutagen**

| Mutagen group | Example | Mode of action |
|---|---|---|
| Alkylating agents | 1-methyl-1-nitrosourea (MNU), 1-ethyl-1-nitrosourea (ENU), methyl methanesulphonate (MMS), ethyl methanesulphonate (EMS), dimethyl sulphate (DMS), diethyl sulphate (DES), 1-methyl-2-nitro-1-nitrosoguanidine (MNNG), 1-ethyl-2-nitro-1-nitrosoguanidine (ENNG) | Add methyl or ethyl groups to DNA bases, leading to mutations during DNA replication. |
| Azide | Sodium azide | Similar action to alkylating agents. |
| Hydroxylamine | Hydroxylamine | Similar action to alkylating agents. |
| Antibiotics | Actinomycin D, mitomycin C, azaserine, streptonigrin | Cause chromosomal changes and can lead to male sterility |
| Nitrous acid | Nitrous acid | Causes deamination, leading to base transitions during DNA replication. |
| Acridines | Acridine orange | Intercalates between DNA bases, causing frameshift mutations |
| Base analogues | 55-bromouracil (5-BU), Maleichydrazide,5-bromodeoxyuridine, 2-aminopurine (2AP). | Substitute for normal DNA bases, causing transitions and tautomerization. |

Source: [33] [30]

## DNA Transposons:

DNA transposons, also known as Class II elements, move from one location in the genome to another via a DNA intermediate, earning them the nickname "jumping genes." These transposons can cause insertions, deletions, and other chromosomal mutations [38], making those promising mutagenic agents for plant mutation breeding.

## Retrotransposons:

Retrotransposons, or Class I transposable elements, are found in all eukaryotes but are absent in prokaryotes [36]. They move through an RNA intermediate and integrate into new genomic sites through DNA produced by reverse transcription of the RNA [39]. Retrotransposons play a crucial role in gene regulation in eukaryotes and can therefore be utilized as gene expression regulators in plant breeding [40].

### Insertion Sequence (IS) Elements:

Insertion sequence elements are segments of bacterial DNA capable of moving within or between genomes [41]. As small pieces of DNA, they represent the simplest type of transposable element [42]. The insertion of IS elements into a genome can cause genomic instability, leading to mutations that can be harnessed in plant mutation breeding [43].

### Crop Improvements Using Mutagens to Create Novel Varieties

The primary approach in mutation-based plant breeding is to enhance well-adapted varieties by modifying one or two key traits [29]. Benefits of mutation breeding include higher yields, early maturity, stress resilience, salt and waterlogging tolerance, and larger seed size [44]. Mutation breeding has produced improved varieties in crops like wheat, rice, and barley. These varieties not only yield more but also exhibit improved quality, earlier maturity, dwarf stature, disease resistance, and reduced toxin content. Additionally, mutation has been used to induce male sterility, which reduces hybrid seed production costs, broadens genetic diversity, and enhances crop adaptation.

**Table 3: Improve Crop Varieties using Mutagen**

| Crop | Botanical name | Mutagen | Trait Improvement | Reference |
|---|---|---|---|---|
| Groundnut | *Arachishypogaea* | X-rays, Gamma rays, Sodium azide. | Large seed, early maturity, seed dormancy, high shelling out-turn, high harvest index, drought tolerance | [45] |
| Soybean | *Glycine max* | Gamma rays | Dwarf, earliness, bacterial leaf pustule resistance | [45] |
| Pigeon pea | *Cajanuscaja* | X-rays, fast neutron, gamma ray, EMS | High yield, large seed, profuse branching, wilt resistance | [45] |
| Cowpea | *Vignaunguiculata* | Gamma rays, DMS | Earliness, green fodder, high yield, fodder | [45] |
| Mustard | *Brassicajuncea* | X-rays, beta rays, gamma rays | Earliness, large seed, high oil, seed coat color | [45] |
| Sesame | *Sesamumindicum* | Gamma rays | High seed retention capability | [46] |
| Maize | *Zeamays* | DMS, DES, EMS | High grain yield and productivity; tolerance to dense sowing; early ripening; drought tolerance; high protein content; high biomass dry matter; shifts in the flowering time; white-color grain; strong stem; altered ear length; increased number of rows | [47] |
| Durum wheat | *Triticum durum* | Gamma rays, EMS | High yield, high productivity; high cold tolerance; short stem; awnlessness; new leaf shape - spherococcum,erectum, compactum, spread rosette | [47] |
| Barley | *Hordeumvulgare* | Gamma ray, UV-C light. | Increased productivity; improved grain characteristics; spike fertility; short and lodging resistant stalks; short vegetation period; high hardness to dip; high cold tolerance, resistance to powdery mildew and to brown, black and stem rust | [47] |
| Cocoa | *Gossypium* | Gamma ray | High yield; length uniformity; high maturity coefficient, shorter vegetative period; high fibre strength | [47] |
| Tobacco | *Nicotiana* | DES, fast neutron | Male sterility, shorter vegetation period, resistance to: Peronosporatabacina, Erysiphecicoracearum, AgrotisIpsilon | [47] |
| Pepper | *Capsicum annum and Capsicum frutescent* | Fast neutron | High growth and yield | [48] |
| Common wheat | *Triticumaestivum* | Gamma rays, NaN3, EMS | High grain yield; high and stable productivity; ecological adaptability, drought and cold tolerance; improved tolerance to lodging and shedding; resistance to brown rust; quality index softening of dough and energy for dough formation | [47] |

**Table 4: Officially released mutant varieties of some Cereals and Pulses in the FAO/IAEA Mutant Varieties Database: (Chemical Mutagens, 2000–2022)**

| Common Name | Variety Name | Mutagen | Country | Date of Release |
|---|---|---|---|---|
| Barley | Gama | Chemical | Ukraine | 2000 |
| Rice | IIyou 3027 | Physical | China | 2000 |
| Rice | IIyou 949 | Physical | China | 2001 |
| Lentil | Binamasur-1 | Chemical | Bangladesh | 2001 |
| Rice | DT22 | Chemical | Viet Nam | 2002 |
| Cowpea | COCP 702 | Physical | India | 2002 |
| Rice | Chiyo-no-mochi | Physical | Japan | 2003 |
| Groundnut | Mutant 28-2 | Chemical | India | 2003 |
| Rice | Asa-tsuyu | Physical | Japan | 2004 |
| Lentil | Binamasur-2 | Physical | Bangladesh | 2005 |
| Mungbean | Binamoong-7 | Chemical | Bangladesh | 2005 |
| Rice | LGC- Jun | Chemical | Japan | 2006 |
| Wheat | Fumai 2008 | Physical | China | 2006 |
| Black gram | DU-1 | Physical | India | 2007 |
| Rice | Miya-yutaka | Physical | Japan | 2007 |
| Chickpea | CM-2008 | Chemical | Pakistan | 2008 |
| Rice | Liangyouhang 2 | Physical | China | 2008 |
| Soyabean | Hefeng 57 | Physical | China | 2009 |
| Maize | Kneja 546 | Chemical | Bulgaria | 2009 |
| Soyabean | Mutiara 1 | Physical | Indonesia | 2010 |
| Soyabean | DT 2008 | Physical | Viet Nam | 2011 |
| Wheat | Leroy | Physical | Ukraine | 2017 |
| Wheat | Deada | Chemical | Ukraine | 2017 |
| Barley | Bima-4 | Physical | Indonesia | 2021 |
| Wheat | Bari Gom-33 | Physical | Bangladesh | 2020 |
| Rice | NHR1 and NHR2 | Physical | Pakistan | 2022 |
| Rice | Dianyou 6 | Physical | China | 2021 |

Source: [49]

**3.3** The economic value of a newly developed mutant variety can be evaluated using several factors. These factors include the area planted with the variety and the proportion of the region's total crop area it represents; increased yields; improved quality; reduced reliance on pesticides and fungicides; water savings due to shorter growth duration and drought tolerance; increased land use efficiency through early maturity, enabling crop rotation; enhanced or intensified cropping systems due to changes in maturity or photoperiod response; better processing quality and product value (such as oil, starch, malt, beer, and whisky); consumer preferences for qualities like flower and foliage color in ornamentals, skin and flesh color in root, tuber, and fruit crops, aroma and glutinous texture in rice, and kernel color in wheat; higher nutritional value, including increased lysine and vitamins, extended oil shelf life, and reduced toxins; higher yields of essential oils; development of new specialty and designer crops; ease of harvest and threshing; increased export revenue; and reduced import needs [50]. Often, the benefits of induced mutations extend beyond the intended phenotypic changes, offering multiple advantages.

## CONCLUSION

Mutation breeding has made significant progress and has become a crucial method for developing mutant varieties. This approach is playing a vital role in generating genetic diversity in crops, leading to improved agricultural outcomes that enhance community livelihoods and contribute to global food and nutritional security. The induction of mutations is particularly important as it allows the identification of new mutations that can improve the nutritional quality of crop plants. With advances in breeding techniques, the development of genetically unique germplasm with increased levels of these and other health-beneficial components is becoming increasingly achievable.

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