

REVIEW ARTICLE

HARNESSING MUTATION BREEDING FOR SUSTAINABLE CROP DEVELOPMENT: FROM PRINCIPLES TO SUCCESS STORIES

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Abstract: Mutation breeding has emerged as a powerful tool in crop improvement, offering a means to generate novel genetic variation beyond what is available in natural germplasm. By inducing mutations through physical and chemical mutagens, breeders have successfully developed improved varieties with enhanced yield, quality, stress resistance, and adaptation traits. Cereals, legumes, oilseeds, horticultural crops, and industrial crops have benefited extensively, with rice, barley, groundnut, soybean, grapefruit, cotton, and vegetatively propagated crops contributing notable success stories. The integration of modern molecular platforms such as TILLING, Mut Map and next-generation sequencing has transformed mutation breeding from a largely random process into a targeted and high-throughput strategy, enabling rapid allele mining and gene discovery. Despite challenges related to large-scale screening, epigenetic instability, linkage drag, and perception issues, advancements in high-throughput phenotyping, predictive breeding, and genomic selection are significantly improving the efficiency of mutant detection and deployment. With growing emphasis on climate resilience and sustainable agriculture, mutation breeding remains a complementary approach to genome editing, capable of creating unique alleles and offering regulatory advantages in many regions. This review highlights the principles, technological innovations, limitations, and success stories of mutation breeding, underscoring its enduring relevance in developing future-ready, sustainable crop varieties.

Keywords: Mutation, Breeding, Crop improvement

INTRODUCTION

Mutation breeding has emerged as a pivotal approach in modern crop improvement, enabling the creation of new genetic variations that are often difficult to obtain through traditional breeding methods. By inducing heritable changes in the DNA sequence using physical, chemical, or biological mutagens, mutation breeding accelerates the development of improved crop varieties with desirable traits such as enhanced yield, disease resistance, abiotic stress tolerance, and superior nutritional quality. The suitability of crops for mutation breeding depends on several factors, including their reproductive behaviour, genetic

background, and mutation frequency. Both genetic and epigenetic mechanisms play crucial roles in determining the stability and expression of induced mutations, influencing the success of trait development.

Advancements in molecular biology, high-throughput screening, and genomic tools have significantly strengthened mutation breeding by facilitating precise detection, characterization, and selection of beneficial variants. Applications of mutation breeding are widespread across cereals, pulses, oilseeds, fruits, and ornamentals, contributing notably to global food and nutritional security. Numerous success stories—such as improved rice, wheat, barley, banana, and groundnut cultivars—

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highlight the practical relevance and commercial impact of this technology. As climate change intensifies stress conditions and threatens agricultural productivity, mutation breeding continues to be a valuable and sustainable strategy for delivering innovative crop improvements to meet the needs of a growing population.

Since the first reports of induced mutations in the 1920s and the post-World War II expansion of radiation facilities, mutation breeding has produced a rich catalogue of mutant phenotypes used directly as cultivars or as sources of useful alleles in conventional breeding programs (Maluszynski *et al.*, 2000). As of recent counts, thousands of mutant varieties (across cereals, legumes, oilseeds, horticultural and industrial crops) have been officially released worldwide and are recorded in the FAO/IAEA Mutant Variety Database (MVD). Mutation breeding remains especially relevant for crops with narrow genetic bases, vegetatively propagated crops, and traits that are difficult to access through conventional recombination. In addition, the complementarity of induced mutations with molecular techniques (TILLING, sequence-based allele mining, and targeted genome editing) has revitalized interest in induced mutagenesis as part of integrated breeding pipelines (Nerkar *et al.*, 2022; Shahwar *et al.*, 2023).

This review presents a comprehensive synthesis of methods, crop suitability, genetic and epigenetic determinants of mutant phenotypes and stability, applications, and case studies of successful mutant varieties, followed by practical recommendations for modern mutation-based programs.

METHODS OF INDUCING MUTATIONS

Physical Mutagens

Physical agents—primarily ionizing radiations (gamma rays from ^{60}Co / ^{137}Cs sources, X-rays, fast neutrons, ion beams) are the most widely used mutagens in plant breeding. Gamma irradiation historically produced the bulk of mutant varieties, while newer techniques (ion beams) generate different mutation spectra (large deletions, chromosomal rearrangements) that can be useful for functional genomics and creating loss-of-function alleles (Mba, 2013). Ion beam mutagenesis is increasingly used for higher mutation density and diverse lesion types (Ghanim, 2024).

Chemical Mutagens

Chemical mutagens such as ethyl methane sulfonate (EMS), ethyl nitrosourea (ENU), sodium azide (NaN_3), and nitrosomethylurea introduce point mutations (mostly GC→AT transitions with EMS) and are valuable for generating allelic series and point mutations useful in reverse genetics (TILLING platforms). Chemical mutagenesis is favored when subtle allelic variation (e.g., enzyme activity modulation) is desired (Mba, 2013; Nerkar *et al.*, 2022).

Biological and Insertional Mutagenesis

Transposable elements, insertional mutagenesis using T-DNA or transposons, and somaclonal variation from tissue culture provide alternative sources of novel variation, often with tagging advantages for gene cloning.

Targeted and Precision Mutagenesis

While traditional random mutagenesis remains important, modern targeted technologies (CRISPR/Cas and other genome editors) provide precise allelic variants. Although CRISPR is not "mutation breeding" in the classical sense, it complements mutation breeding by enabling precise recreation of beneficial induced alleles or rapid functional validation of mutants (Nerkar *et al.*, 2022; Shahwar *et al.*, 2023).

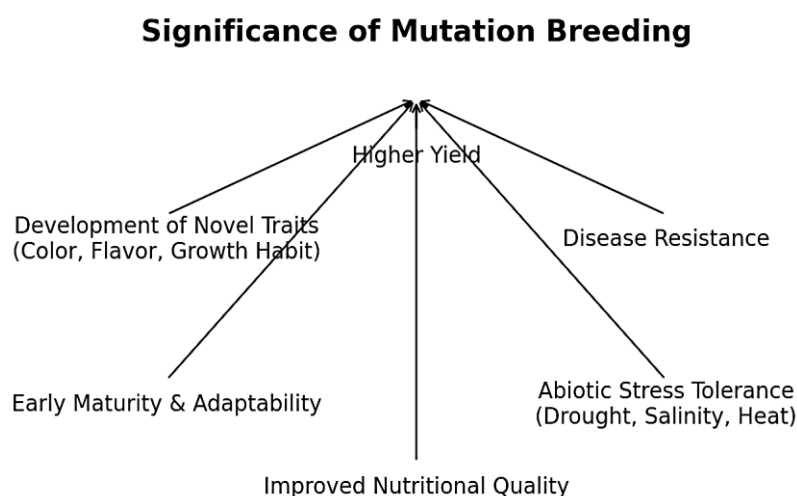


Figure 1: Importance of Mutation Breeding in Crop Improvement for Sustainable Agriculture. Mutation breeding contributes significantly to crop improvement by enhancing yield, strengthening disease resistance, increasing tolerance to abiotic stresses, enabling early maturity, improving nutritional quality, and generating novel traits for better adaptability and performance.

Screening, Selection and Validation Strategies

A major bottleneck in mutation breeding is the efficient identification of desirable mutants within large M1/M2 populations. To overcome this, several strategies are adopted, including phenotypic screening under controlled and stress environments such as drought, salinity, and disease inoculation, allowing the selection of visible beneficial traits. Molecular screening approaches like TILLING and targeted amplicon sequencing help detect specific point mutations in candidate genes, improving precision in mutant detection. High-throughput phenotyping (HTP) technologies-including imaging tools, UAV-based monitoring, and automated screening platforms-further enhance the rapid assessment of physiological and architectural traits across large populations. Additionally, backcrossing and trait validation steps ensure the genetic stability and agronomic suitability of selected mutants for commercial cultivation (Ghanim, 2024). Advances in next-generation sequencing (NGS) alongside TILLING have significantly accelerated allele discovery in chemically mutagenized populations, ultimately reducing the time required for trait-specific variety development and release (Mba, 2013; Nerkar *et al.*, 2022).

Crops Most Suitable for Mutation Breeding

Mutation breeding is applicable across diverse crop groups, but its success and adoption vary depending on biological and agronomic characteristics. Cereals such as rice, barley, and wheat have historically seen the highest number of mutant varieties, mainly due to large cultivation area, easy seed propagation, and the presence of distinct phenotypic traits like semi-dwarfism, maturity period, and grain quality. Rice alone contributes a major portion of the mutant variety database (Mba, 2013). Similarly, legumes and oilseeds including groundnut, soybean, and rapeseed have benefitted significantly from mutation breeding, leading to improvements in oil quality, disease resistance, and growth duration. Horticultural crops such as grapefruit, ornamental plants, and fruit trees have also been improved through induced mutations, particularly for traits like fruit colour, seed lessness, and modified flowering behaviour. Well-known examples include the grapefruit mutants 'Star Ruby' and 'Rio Red' (FAO/IAEA; Mba, 2013). In vegetatively propagated crops like cassava, banana, potato, and sugarcane, mutation breeding combined with *in vitro* techniques has contributed to the development of early-maturing, virus-resistant, and modified starch-type cultivars (Ghanim, 2024). Additionally, industrial crops such as cotton and other fibre crops have seen fibre quality improvements through induced mutants, benefitting the textile industry. Overall, the suitability of mutation breeding is strongly influenced by crop biology including life cycle duration, mode of

propagation, feasibility of mutagenesis, and availability of efficient screening techniques.

GENETIC BASIS OF MUTANT TRAITS

Types of Mutations and Effects

Induced mutations range from single nucleotide polymorphisms (SNPs) to large deletions, inversions, and chromosomal rearrangements. The phenotypic effect depends on mutation type (loss-of-function vs gain-of-function), gene redundancy, and interaction with the genetic background. EMS tends to produce point mutations (useful for TILLING), whereas gamma rays and neutrons often cause larger structural changes (Mba, 2013).

Dominance, Epistasis and Background Effects

Mutant phenotypes may be recessive, dominant or partially dominant. Many useful traits (e.g., semi-dwarfism) are often semi-dominant and readily selected in early generations, while complex traits (yield, tolerance) involve epistasis and require multi-generation evaluation and backcrossing to elite backgrounds (Figure 1) (Mba, 2013).

Mutation Rate and Spectrum

Mutation spectrum is mutagen-specific. EMS produces high density of point mutations (good for saturation mutagenesis), whereas ion beams provide diverse lesion types. Mutation rate optimization (dosage, exposure time) balances survival and mutation frequency; LD50 estimation is standard practice (Shahwar *et al.*, 2023).

Epigenetic Factors: Role and Application in Mutation Breeding

Epigenetics in Plants-Overview

Epigenetic modifications (DNA methylation, histone modifications, chromatin remodelling, small RNAs) modulate gene expression without altering DNA sequence and can be heritable across generations in plants (Tonosaki *et al.*, 2022; Tirnaz & Batley, 2019). Environmental stress, tissue culture, and mutagenic treatments can induce epigenetic changes that affect phenotype.

Epigenetic Variation as a Breeding Resource

Epigenetic variants (epialleles) provide additional heritable variation that can be harnessed for traits such as stress tolerance, flowering time, and metabolic traits. The concept of "epibreeding" proposes selection of stable epialleles or inducing beneficial epigenetic states through treatments (e.g., demethylating agents) or selection after tissue culture (Gupta *et al.*, 2022; Tonosaki *et al.*, 2022).

Interaction Between DNA Mutation and Epigenetics

Mutagenesis can induce both DNA sequence changes and epigenetic modifications. Furthermore, some induced mutations may affect genes involved in epigenetic regulation (methylases, chromatin factors), resulting in transgenerational epigenetic

effects. Conversely, epigenetic changes can modulate the expression of induced mutant alleles, altering penetrance and expressivity (Cao *et al.*, 2024; Varotto *et al.*, 2020).

Success Stories and Representative Varieties

Successful mutant varieties documented in the FAO/IAEA Mutant Variety Database (MVD) and literature. These examples illustrate the range of traits and crops improved via mutation breeding (Table.1).

Table 1. Representative mutant varieties released

S. No.	Crop	Variety / Mutant Line	Key Improved Trait(s)	Mutagen Used	Country (Year)	References
1	Grapefruit (<i>Citrus × paradisi</i>)	Rio Red	Deep red flesh, improved juice quality	Thermal neutron / irradiation	USA (1976)	FAO/IAEA
2	Groundnut (<i>Arachis hypogaea</i>)	TG-26	Disease resistance, improved agronomy	Gamma rays / EMS	India	RCA brief
3	Rice (<i>Oryza sativa</i>)	Multiple commercially released mutants	Semi-dwarfism, yield, quality traits	Gamma rays / EMS	Asia, worldwide	Ma <i>et al.</i> 2021
4	Barley (<i>Hordeum vulgare</i>)	Multiple mutants (e.g., malt quality)	Quality improvement, lodging resistance	Gamma irradiation	Europe/Asia	Maluszynski <i>et al.</i> , 2000 IAEA
5	Wheat (<i>Triticum aestivum</i>)	Dwarf & rust-resistant mutants	Semi-dwarfism, disease resistance	Gamma rays / chemicals	Global	FAO/IAEA; Ma 2021
6	Satsuma Mandarin (<i>Citrus unshiu</i>)	Gwonje-early	Early maturity	Gamma irradiation	Korea (2024)	Eun <i>et al.</i> , 2024
7	Sugarcane	Mutant clones	High sugar yield, ratooning ability	Physical / chemical (Radiation, EMS)	Various	Barrientos-Alfaro <i>et al.</i> , 2025
8	Banana	Pisang SPM-130	Fusarium wilt resistance	EMS	Malaysia	Oladosu <i>et al.</i> 2016
9	Chickpea	Pusa 408 / Pusa 547	Disease resistance	Gamma ray / EMS	India	Oladosu <i>et al.</i> 2016

Successful Mutant Varieties

Rio Red and Star Ruby Grapefruit

Rio Red and Star Ruby (red-fleshed grapefruit cultivars) exemplify the horticultural impact of induced mutation. Thermal neutron and other radiation treatments produced fruit with deeper red flesh and improved juice quality, traits that became commercially valuable in the US and global markets. These cultivars illustrate how bud sports and irradiated nucellar lines can produce commercially dominant phenotypes (Da Graça *et al.*, 2004; Louzada & Del Rio, 2021).

Mutant Rice Varieties

Rice has the highest number of mutant entries in MVD. Mutant selections produced semi-dwarf phenotypes, early-maturing lines, and grain quality variants. These mutants have been used directly or as parents in breeding programs to improve plant architecture and adaptation (Mba, 2013).

Groundnut Mutants (TG-26 and Others)

Groundnut mutant varieties (e.g., TG-26) developed in India improved disease resistance and agronomic performance; multiple mutant groundnut cultivars have been released and cultivated widely. These cases demonstrate mutation breeding utility in improving legumes with narrow genetic bases.

Integration with Molecular Tools and Modern Breeding

Modern molecular tools have significantly enhanced the precision and efficiency of mutation breeding. TILLING (Targeting Induced Local Lesions in Genomes) is a widely used reverse genetics approach that screens mutant populations to identify allelic variations in specific genes without requiring transgenic methods, thus making it valuable for functional genomics and breeding programs (Mba, 2013). Additionally, high-throughput sequencing-based strategies such as MutMap and MutMap-G enable rapid identification of causal polymorphisms

in mutant lines, allowing breeders to associate mutations directly with target traits. Further advancement comes through integrated hybrid pipelines, where induced mutations generate novel alleles that are subsequently introgressed into elite cultivars using marker-assisted selection (MAS) or genomic selection, ensuring both efficiency and precision in trait improvement (Nerkar *et al.*, 2022; Shahwar *et al.*, 2023). Collectively, these tools shorten the breeding cycle, accelerate the path from mutation induction to varietal release, and facilitate targeted allele mining for complex quantitative traits.

Achievements of Mutation Breeding

Higher yield, earliness, stress resilience, salt tolerance, water logging tolerance, and bold seed size are some of the benefits of mutant breeding. In wheat, rice, and barley, improved varieties have been generated by mutation breeding. In addition to high yield, improved quality, earliness, dwarfness, disease resistance, and reduced toxin content have all been created in numerous crop varieties. Mutation has also been employed to induce male sterility, which lowers the cost of hybrid seed production, increases the range of genetic variety, and aids crop adaptation. There have been 2252 mutant variants developed in plant species across the world. 1585 have been released directly, while 667 have been released using mutants in hybridization. 1700 mutant variants have been introduced in seed propagated crops and 552 in vegetatively propagated species, out of a total of 2252. Rice (434 mutant variants) has the most mutant varieties created among seed propagated species, followed by barley (269), and wheat (222). Radiations have resulted in the development of maximum varieties.

EMS resulted in the production of maximal mutant variations among chemical mutagens [the most effective strategies for creating genetic variation and identifying critical regulatory genes for economically relevant features in crop development. Recent developments in genomics technology have resulted in a proliferation of genomic techniques in applied breeding, notably mutational breeding. Plant breeding has employed mutagenesis, or the act of creating mutations within an organism's genome. Induced mutagenesis and associated breeding tactics can improve quantitative and qualitative qualities in crops in a fraction of the time it takes to do so with traditional breeding. The global effect of mutation breeding derived agricultural types highlights mutation breeding's promise as a versatile and practical approach to any crop.

Challenges and Limitations

Despite its wide application and notable achievements, mutation breeding is accompanied by several challenges that constrain its full potential. One of the major limitations lies in the need for intensive screening, as selecting promising mutants from vast M2 populations demands substantial time, labor, and advanced phenotyping capacities. Another

concern is linkage drag and pleiotropy, where radiation-induced large chromosomal deletions may inadvertently remove beneficial linked genes or cause undesirable effects. Moreover, epigenetic instability arising from somaclonal variation or stress-related epigenetic changes can lead to inconsistent trait expression across generations. Public perception also plays a role, as mutation breeding, although non-transgenic, is sometimes misunderstood and may face resistance in certain regions. Additionally, intellectual property complexities and benefit-sharing issues can limit the accessibility of mutant-derived germplasm in global markets. Overcoming these limitations will require greater adoption of high-throughput phenotyping, strategic integration of genomic and molecular tools, and improved communication to build trust and awareness regarding the safety and advantages of induced mutants (Varotto *et al.*, 2020).

Practical Recommendations for Breeders

For effective implementation of mutation breeding, breeders should adopt strategic approaches that enhance mutation efficiency, screening accuracy, and stability of selected variants. First, conducting dose-response (LD50) assays is essential to determine the optimal mutagen concentration for each crop and genotype, ensuring sufficient mutation induction while minimizing lethal effects. Integrating random mutagenesis techniques such as EMS or gamma irradiation with targeted identification tools like TILLING and next-generation sequencing can greatly improve the ability to discover beneficial alleles, particularly for gene-specific improvement. High-throughput phenotyping systems and multi-environment evaluations should be utilized to efficiently detect mutants for complex and quantitative traits. Additionally, continuous monitoring of epigenetic stability is crucial, especially for mutants developed through tissue culture, where somaclonal variation can influence trait expression. Finally, breeders are encouraged to utilize resources such as the FAO/IAEA Mutant Variety Database (MVD) to locate existing mutants, prevent duplication of work, and facilitate the use of validated donor material for breeding pipelines (IAEA MVD, 2024).

Future Prospects

Mutation breeding is expected to remain a valuable and complementary approach alongside modern genome editing techniques. Its continued relevance is rooted in its ability to generate novel alleles that may not exist in natural gene pools, providing a unique resource for breeding programs, particularly in regions where stringent regulations limit the use of gene editing technologies. When combined with advanced molecular tools such as deep sequencing, TILLING, and MutMap methods, induced mutations can be identified, characterized, and deployed much faster than in traditional workflows. Emerging strategies like epigenetic selection, or epibreeding,

further expand the potential of mutation breeding by exploiting heritable epigenomic variation for traits related to stress tolerance and adaptability (Tonosaki *et al.*, 2022; Tirnaz & Batley, 2019). Moreover, the integration of mutation breeding with high-throughput phenotyping platforms, predictive breeding approaches such as genomic selection, and precision mutagenesis tools like ion beam irradiation offers a forward-looking path for accelerating the development of climate-resilient cultivars (Ghanim, 2024).

CONCLUSIONS

Mutation breeding has a proven track record and continues to be a practical and cost-effective approach to expand breeding variation. Its strengths lie in generating novel alleles, improving vegetatively propagated and under-resourced crops, and complementing molecular breeding. The future will see deeper integration of induced mutagenesis with high-resolution genomics, epigenomics, and phenomics, enabling faster delivery of resilient, high-performing varieties for food security and climate adaptation.

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