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CRISPR and Beyond: The Future of Induced Mutagenesis in Plants

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ABSTRACT

Induced mutagenesis has evolved significantly from the use of random physical and chemical mutagens to the adoption of highly precise genome editing technologies, notably CRISPR/Cas systems. This review presents a comprehensive and critical analysis of the methodologies utilized in induced mutagenesis, with a focus on recent advancements, trait-improvement applications, and emerging tools such as base editing and prime editing. Key challenges, including the detection of off-target mutations, regulatory complexities, and the need for comprehensive functional annotation of genes, are highlighted through comparative analyses and functional evaluations. The integration of machine learning approaches and the development of efficient in planta delivery systems are proposed as promising future directions to enhance precision and scalability. This rapidly evolving landscape holds transformative potential for sustainable crop improvement and global food security.

KEYWORDS

Induced mutagenesis, CRISPR/Cas9, chemical and physical mutagens, genome editing, trait improvement, base editing, prime editing, epigenome editing, off-target effects

1. Introduction

A fundamental component of plant breeding, induced mutagenesis makes it easier to create the genetic variability needed for crop adaptation and improvement. Breeders have created new traits that might not have developed through natural variation alone by purposefully changing the plant genome by applying physical or chemical agents. The development of high-yielding, disease-resistant, and stress-tolerant cultivars of a variety of crops has been greatly aided by this strategy, improving global food security (Oladosu et al., 2016). In order to cause random mutations in plant genomes, physical mutagens such as gamma and X-rays as well as chemical agents such as sodium azide and ethyl methanesulfonate (EMS) have been used extensively in the past. Even though these traditional methods have produced some noteworthy results, such as the creation of more than 3,000 mutant varieties that have been formally released globally, they have drawbacks such as poor precision, a large number of background mutations, and the requirement for time-consuming phenotypic screening (Shu et al., 2012). Furthermore, the randomness of these mutations makes gene-function research more difficult and prevents the quick adoption of desired traits. A paradigm shift in the study of mutagenesis has been brought about by the development of genome editing technologies, especially CRISPR/Cas systems. By enabling targeted, site-specific DNA sequence modifications, these tools greatly improve the effectiveness, precision, and predictability of genetic interventions. Among these, the CRISPR/Cas9 platform has become well-known because of its versatility across plant species, ease of use, and multiplexing ability. It has sped up functional genomics research and trait development pipelines by enabling accurate knockouts, insertions, and base edits (Chen et al., 2019).

The progression of induced mutagenesis from conventional, non-targeted methods to contemporary, precision-based genome editing tools is critically assessed in this review. It outlines new scientific findings, talks about the drawbacks and difficulties of existing approaches, points out areas for further study, and suggests ways to combine induced mutagenesis with other cutting-edge technologies in plant breeding and agricultural development.

2. Traditional Induced Mutagenesis: Scope and Limitations

In plant breeding, traditional induced mutagenesis has long been a crucial technique that allows for the creation of novel genetic variation outside the bounds of natural recombination. The method uses chemical and physical mutagens, such as sodium azide (NaN_3), methyl nitrosourea (MNU), and ethyl methanesulfonate (EMS), as well as gamma rays, X-rays, and fast neutrons, to cause random mutations throughout the genome. These mutagens cause deletions, chromosomal rearrangements, and point mutations, which frequently produce changed phenotypes that can be used to improve crops.

Traditional mutagenesis has a number of drawbacks due to its inherent randomness and lack of targeting, despite its substantial contributions. These consist of:

i) Low mutation specificity: Unwanted background mutations frequently result from mutations that happen throughout the genome without control over target loci.

ii) Labour-intensive screening: Extensive phenotypic evaluation and sizable mutant populations are necessary for the identification and selection of advantageous mutations.

iii) Limited throughput: Rapid trait integration and high-throughput gene-function studies are not supported by classical methods.

iv) Unpredictable effects: Some induced mutations can cause harmful or fatal phenotypes, which makes subsequent breeding attempts more difficult.

v) Biosafety and regulatory considerations: Even though mutant lines aren't considered genetically modified organisms (GMOs), they might still need to be carefully assessed before being sold.

By allowing reverse genetics techniques to detect mutations in particular genes, innovations such as TILLING (Targeting Induced Local Lesions IN Genomes) have increased the usefulness of conventional mutagenesis (Kurowska et al., 2011). In contrast to contemporary genome editing methods, the entire procedure is still laborious and imprecise. Although conventional mutagenesis established the groundwork for contemporary crop improvement, its drawbacks highlight the necessity of accurate, effective, and focused techniques. While some of these difficulties have been somewhat alleviated by the combination of conventional mutagenesis with molecular marker-assisted selection and next-generation sequencing, the field must advance toward more sophisticated instruments such as CRISPR/Cas systems.

Table 1. Comparison of Traditional Mutagenesis Agents

Mutagen Type	Examples	Mechanism	Crops Improved	Limitations
Physical	Gamma rays, X-rays	Induces double-strand breaks, chromosomal rearrangements	Rice, wheat, barley	Randomness, off-target effects, limited precision

Chemical	EMS, Sodium azide	Alkylation of bases leading to point mutations	Tomato, lentil, soybean	High screening burden, pleiotropic effects, environmental and health concerns

Critical Analysis: The expansion of crops' genetic base has been greatly aided by traditional induced mutagenesis. Over 3,300 mutant varieties have been successfully produced using it worldwide (IAEA, 2021). These methods are particularly helpful for crops with limited genomic resources and in developing nations because they are affordable and do not require prior knowledge of gene function. But they come with a number of disadvantages**.** Large-scale phenotypic screening is necessary to isolate advantageous traits because mutations are random, making outcome control challenging. According to Mba et al. (2010), there is also a chance of harmful mutations and unforeseen pleiotropic effects, which could jeopardise plant fitness or yield stability. Because of this, even though conventional mutagenesis is still a useful technique, its drawbacks make the creation and use of more accurate genome editing technologies necessary.

3. The Shift to Precision: Genome Editing Technologies

With the development of genome editing technologies, induced mutagenesis has undergone a radical change from random to targeted alterations. Modern genome editing tools such as Zinc Finger Nucleases (ZFNs), Transcription Activator-Like Effector Nucleases (TALENs), and proteins linked to Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) enable precise changes at particular genomic loci, in contrast to traditional mutagens. Double-strand breaks at specific locations were made possible by ZFNs and TALENs, which introduced tunable DNA-binding domains fused with nuclease domains, albeit with design complexity and financial limitations (Voytas, 2013).

The most adaptable and widely available genome editing platform is CRISPR/Cas9 technology, which was derived from bacterial immune defence mechanisms. It uses a single-guide RNA (sgRNA) to guide the Cas9 nuclease to a specific DNA sequence, causing a double-strand break that can be fixed by homology-directed repair (HDR) or non-homologous end joining (NHEJ). Its broad application in plant systems such as rice, maize, and wheat has been made possible by its ease of use, high efficiency, and multiplexing ability (Jaganathan et al., 2018).

With the advent of CRISPR/Cas technology, site-specific mutagenesis replaced random mutagenesis, enabling accurate edits at predefined loci. With the help of a guide RNA (gRNA), the Cas endonuclease is guided to the target DNA sequence, where it creates a double-strand break (DSB), which is then fixed by NHEJ or HDR (Chen et al., 2019). CRISPR/Cas systems have made it possible to efficiently create modified varieties of crops such as barley and wheat that have characteristics such as changed flowering time, increased disease resistance, and improved stress tolerance (Koeppel et al., 2022).

A transgene-free method called ribonucleoprotein (RNP)-mediated mutagenesis has become more well-liked due to its regulatory benefits. RNPs, which minimize off-target effects and do not require DNA integration, are pre-assembled Cas proteins with guide RNAs that are delivered directly into plant cells (Becker et al., 2022).

Table 2. Comparison of Genome Editing Technologies

Genome Editing Tool	Precision	Complexity	Plant Systems Applied	Limitation
ZFNs	High	High	Arabidopsis, maize	Expensive, labor-intensive
TALENs	High	Moderate	Rice, tobacco	Delivery and design complexity
CRISPR/Cas9	Very High	Low	Wheat, rice, tomato	Off-target potential, PAM dependency

Critical Analysis: The versatility and ease of use of CRISPR/Cas9 have greatly increased the mutagenesis toolkit. But reducing off-target effects, increasing HDR efficiency, and editing complex genomes, particularly in polyploid species, remain difficult tasks. Some of these constraints are being addressed by innovations such as RNP-based delivery, high-fidelity Cas variants, and next-generation editing platforms such as prime editing, which are opening the door to more sophisticated genome engineering.

4. CRISPR and Doubled Haploids: A Game-Changer in Barley

The combination of microspore-derived doubled haploid (DH) technology and CRISPR-based genome editing has led to a revolutionary breakthrough in plant breeding, especially in crops such as barley (Hordeum vulgare). In order to create fully homozygous, modified plants in a single generation, this combined strategy allows for meticulous, site-directed mutagenesis during the early phases of haploid development, followed by spontaneous or induced chromosome doubling. By effectively editing target genes during DH generation in barley, Hoffie et al. (2023) showcased this novel approach, thereby reducing the traditional multi-year breeding cycle to a single step.

This method is useful because it can quickly correct mutations, which removes the need for backcrossing or repeated selfing, which is usually necessary to reach homozygosity in conventional breeding. Recessive traits, which would otherwise be hidden in heterozygous states and require extra generations for trait expression, benefit greatly from this. Breeders can produce homogeneous populations of modified plants that are instantly appropriate for phenotypic assessment, functional genomics, or commercial deployment by directly causing mutations in microspores.

Additionally, this method represents a paradigm shift in the introduction and stabilization of genetic traits and improves plant breeding efficiency, speed, and precision. Additionally, it offers a sustainable model for high-throughput trait development by lowering the time and resource inputs connected to traditional breeding pipelines. The method has a lot of potential for barley as well as other cereals and crops such as wheat, maize, and rapeseed that have well-established microspore culture systems.

Combining CRISPR with DH systems not only speeds up trait fixation but also makes it easier to validate gene edits functionally in a genetically homogeneous background, which improves genotype-to-phenotype relationships. In the context of climate-resilient and nutritionally enhanced cultivars, the combined technique of in vitro androgenesis and genome editing is expected to become a standard tool in next-generation breeding programs as their efficiency increases.

Overall, Hoffie et al.'s study from 2023 is a great example of how contemporary biotechnological tools can work together to improve genetic gain, expedite breeding pipelines, and explore new areas of crop

improvement research.

Table 3. Comparative Analysis: Traditional vs. CRISPR-Based Mutagenesis

Feature	Traditional Mutagenesis	CRISPR-Based Editing
Specificity	Low	High
Efficiency	Moderate	High
Regulatory Hurdles	Low	Variable
Public Acceptance	High	Mixed

5. Functional Applications of Modern Mutagenesis

The use of functional mutagenesis in crop improvement has significantly increased since the development of precise genome editing technologies, especially CRISPR/Cas systems. Modern techniques enable the targeted manipulation of particular genes linked to agronomic traits, in contrast to traditional mutagenesis, which results in random genetic changes. Crop varieties with increased resistance to biotic and abiotic stressors, increased yield, and superior nutritional quality have all been made possible by this precision.

5.1 Biotic Stress Resistance

The ability to engineer resistance to diseases and pests has been particularly effective with modern mutagenesis. The knockout of *MLO* (Mildew Resistance Locus O) genes in wheat (*Triticum aestivum*) by CRISPR/Cas9 is among the best-known examples. Without influencing plant growth or yield, the targeted disruption of the *MLO* genes, which are negative regulators of resistance against powdery mildew, has produced long-lasting resistance (Wang et al., 2014). This approach has the potential to be applied to other cereal crops and is a sustainable substitute for chemical fungicides.

5.2 Abiotic Stress Tolerance

Crop productivity is severely hampered by abiotic stressors such as salinity, drought, and extreme temperatures. Targeted changes that improve resilience to stress have been made possible by genome editing. For instance, rice (*Oryza sativa*) exhibited markedly enhanced salt tolerance in field conditions after *OsRR22*, a cytokinin response regulator gene, was knocked out using CRISPR/Cas9 (Zhang et al., 2019). DST, OsNAC6, and additional regulatory genes implicated in drought and oxidative stress responses have been the focus of similar initiatives.

5.3 Yield and Quality Improvement

Additionally, genome editing makes it easier to enhance grain quality and yield-related characteristics. The *Waxy (Wx)* gene, which regulates amylose synthesis in rice endosperm, has been precisely altered through the use of base editing, a sophisticated type of CRISPR/Cas technology. Rice lines with lower amylose content were produced through targeted base substitution, improving grain eating and cooking quality without sacrificing yield (Li et al., 2020). For the development of traits focused on consumers and the market, such precise, non-transgenic modifications hold particular promise.

Table 4: Summary of functional applications of Modern Mutagenesis

Application Area	Target Gene(s)	Trait Improved	Editing Technique	Reference
Biotic Stress Resistance	*MLO*	Powdery mildew resistance in wheat	CRISPR/Cas9 knockout	Wang et al., 2014
Abiotic Stress Tolerance	*OsRR22*	Salt tolerance in rice	CRISPR/Cas9	Zhang et al., 2019
Yield & Quality Improvement	*Waxy*	Grain quality in rice	Base editing	Li et al., 2020

Figure 1: Modern Mutagenesis Workflow

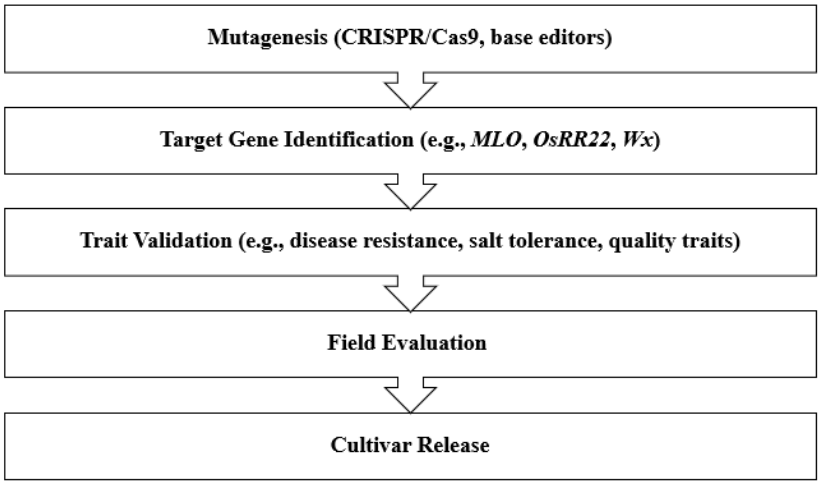

Figure 1. Modern mutagenesis workflow in plant breeding. The process begins with the selection of an appropriate mutagen—physical, chemical, or molecular, followed by application to plant material. Subsequent steps include high-throughput screening for mutations, validation of desired traits, and fixation through breeding techniques such as doubled haploidy. Stable and beneficial mutations are then evaluated in field trials before regulatory approval and cultivar release. Advanced genome editing technologies such as CRISPR, base editing, and prime editing have added precision and efficiency to each stage of this workflow.

6. Emerging Innovations in Genome Editing Technologies

Beyond the capabilities of conventional CRISPR/Cas9-mediated gene knockout systems, modern plant mutagenesis is experiencing a revolutionary expansion. While site-specific double-strand breaks (DSBs) made possible by CRISPR/Cas9 have revolutionised genome editing, the development of next-generation editing platforms has been prompted by the technology's dependence on cellular DNA repair mechanisms and potential for off-target effects. These new developments, base editing, prime editing, and epigenome editing, open up previously unattainable paths in plant functional genomics and trait engineering by providing unprecedented levels of control, flexibility, and precision.

6.1 Base Editing

Without causing double-strand breaks (DSBs) or needing donor DNA templates, base editing is a next-generation genome editing technique that enables the direct, irreversible conversion of one DNA base into another. In order to chemically alter particular bases at specific genomic locations, this technique uses protein complexes that have been engineered to couple a catalytically impaired Cas9 (dCas9 or nCas9) with a deaminase enzyme. Adenine base editors (ABEs), which change A•T base pairs to G•C, and cytosine base editors (CBEs), which change C•G base pairs to T•A, are the two primary classes (Komor et al., 2016; Gaudelli et al., 2017). There is less chance of off-target effects and undesired insertions or deletions (indels) because these exact single-nucleotide conversions are carried out with little disturbance to the surrounding DNA sequence.

Base editing has shown itself to be an effective technique for introducing advantageous allelic variants linked to significant agronomic traits in plant systems. For example, it has been demonstrated that base editing-induced mutations in the ALS (acetolactate synthase) gene confer herbicide resistance in a number of crops, improving weed control and minimising crop damage. Similar to this, base editing has been utilised to enhance grain quality characteristics such as amylose content and aroma by making targeted edits in genes such as Waxy and BADH2, as well as to create disease-resistant cultivars by altering susceptibility genes.

The effectiveness of base editing in both model and non-model crops, including polyploid species, where accuracy is crucial and conventional breeding techniques are frequently laborious and ineffective, is one of its main benefits. Base editing is ideal for allelic engineering and fine-tuning complex traits because it prevents the formation of DSBs, which reduces the likelihood that DNA repair pathways will be activated and cause unpredictable mutations. Moreover, base editing produces modifications that are very similar to natural point mutations, which could make regulatory approval easier in some jurisdictions that separate genome-edited crops from genetically modified organisms (GMOs).

Continuous advancements in editing effectiveness, deaminase activity, delivery methods, and specificity will broaden the technology's uses in functional genomics, sustainable agriculture, and crop improvement. In the end, base editing is a crucial part of precision breeding techniques meant to address global issues with food production, climate resilience, and nutritional security.

Table 5: Summary of Base Editing Technologies and Their Applications in Crop Improvement

Type of Base Editor	**Base Conversion**	**Enzyme Used**	**Mechanism**	**Key Applications in Plants**	**Advantages**
Cytosine Base Editor (CBE)	C•G → T•A	Cytidine deaminase (e.g., APOBEC1) + nCas9/dCas9	Deaminates cytosine to uracil, which is read as thymine during replication	Herbicide tolerance (e.g., *ALS* gene) Disease resistance-Quality trait improvement	Precise editing without DSBs Lower indel formation-Applicable to diverse crops
Adenine Base Editor	A•T → G•C	Adenosine deaminase (e.g., evolved TadA) +	Deaminates adenine to inosine, which is	Trait enhancement in cereals	High specificity

Type of Base Editor	Base Conversion	Enzyme Used	Mechanism	Key Applications in Plants	Advantages
(ABE)		nCas9	interpreted as guanine	(e.g., *OsSPL14* in rice) Alteration of metabolic pathways	Stable single nucleotide conversion Low risk of genomic instability

6.2 Prime Editing

With its unparalleled precision and versatility, prime editing is a revolutionary development in the field of genome engineering. This technology, created by Anzalone et al. (2019), allows for all 12 types of point mutations, targeted insertions, and deletions without the need for donor DNA templates, double-strand breaks (DSBs), or the cell's homologous recombination pathways. A fusion protein of a Cas9 nickase (nCas9) and a reverse transcriptase, guided by a specially created prime editing guide RNA (pegRNA), is used to carry out prime editing. The pegRNA has a template sequence that encodes the intended genetic alteration in addition to guiding the nCas9 to the precise target location. The desired edit from the pegRNA is copied into the genome by the reverse transcriptase after the target site is nicked, making the process highly programmable and flexible.

The ability of prime editing to make precise changes at almost any location, including ones that are challenging to alter with base editors or conventional CRISPR/Cas9 systems, is one of its main advantages. It has proven particularly useful for engineering allelic variants and fixing harmful point mutations without adding exogenous sequences. Prime editing has been successfully used in plants to modify regulatory elements, correct point mutations, and induce herbicide resistance, according to proof-of-concept studies conducted on rice and wheat (Lin et al., 2020). These results point to a great deal of promise for accurate crop trait improvement, particularly when PAM availability or editing scope limit the use of conventional editing techniques.

Prime editing in plant systems is still in its infancy and faces a number of technical obstacles, despite its potential. These include optimising pegRNA length and structure to guarantee stable expression and precise editing, delivering large editing complexes into plant cells efficiently, especially in resistant species, and varying editing efficiency based on chromatin accessibility and cellular repair machinery. Additionally, the novelty of the platform limits off-target evaluations, and editing efficiency in many plant species is still lower than that seen in mammalian systems.

However, it is anticipated that continued work in pegRNA engineering, increased reverse transcriptase fidelity, and improved delivery systems (including viral vectors, nanoparticles, and plant transformation systems) will greatly expand the use of prime editing in crop improvement initiatives. It is a priceless addition to the genome editing toolbox because of its exceptional capacity to produce accurate, consistent, and scar-free edits. This is especially true for uses in precision breeding, synthetic biology, and trait stacking in both important and underutilised crops.

Table 6: Overview of Prime editing in Plants

Feature	**Prime Editing**
Mechanism	Cas9 nickase fused to reverse transcriptase guided by pegRNA
Type of Edit	Point mutations, small insertions, deletions, all 12 base conversions
DSBs Required	No
Donor Template Needed	No
Precision	Very high
Efficiency in Plants	Moderate; varies with pegRNA and target locus
Limitations	Delivery complexity, pegRNA design, lower current efficiency
Applications	Precise trait correction, regulatory element modification

6.3 Epigenome Editing

The goal of epigenome editing, a new field in functional genomics and plant biotechnology, is to change gene expression without changing the underlying DNA sequence. Epigenome editing uses catalytically inactive or "dead" Cas9 (dCas9) fused to chromatin-modifying enzymes to precisely control gene activity, in contrast to conventional genome editing tools such as CRISPR/Cas9 that introduce permanent genetic changes. Histone acetyltransferases (such as p300) can activate gene expression, histone demethylases (such as LSD1) can eliminate repressive chromatin marks, and DNA methyltransferases (such as DNMT3A) or demethylases (such as TET1) can either repress or reactivate gene expression at particular loci (Gallego-Bartolomé, 2020).

Applications where temporary or conditional control is desired will find this non-mutagenic, programmable regulation of gene expression especially appealing since it provides a flexible and reversible method of modifying plant characteristics. For example, epigenome editing can be used to modify secondary metabolite pathways for improved nutritional or defence traits, target flowering pathway regulators (e.g., FLC, FT), or fine-tune plant responses to abiotic stresses such as drought or salinity by controlling stress-responsive genes. Furthermore, in certain jurisdictions, such modifications might not be subject to stringent GMO regulations because no foreign DNA is permanently integrated and no DNA sequence is altered, potentially providing regulatory flexibility.

Proof-of-concept studies have shown that dCas9 fusions can target DNA methylation or histone modification at particular loci in plant systems, resulting in changes in gene expression and related phenotypes. For many crop species, however, the technology is still in its infancy because of issues with delivery efficiency, the temporary nature of some changes, and a lack of knowledge regarding epigenetic inheritance and stability across generations.

However, the use of epigenome editing for crop improvement has a bright future due to the quick advancements in synthetic transcriptional systems, single-cell chromatin profiling, and plant epigenomics. This strategy will be even more useful in metabolic engineering, precision agriculture, and sustainable crop design when combined with inducible or tissue-specific promoters, optogenetic

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tools, and high-resolution epigenetic maps.

Figure 2: Mechanism of Epigenome Editing Using dCas9 Fusions

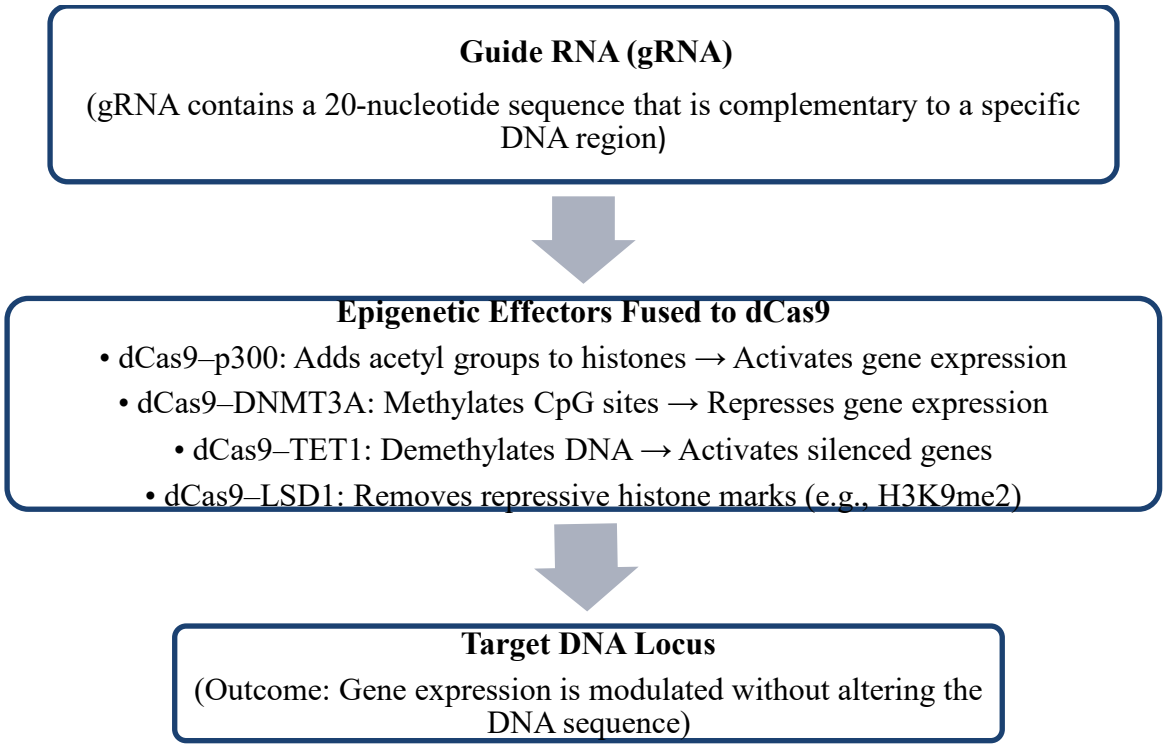

Figure 2: Mechanism of dCas9-based epigenome editing. A catalytically inactive Cas9 (dCas9) is guided by a single-guide RNA (gRNA) to a specific genomic region. By fusing dCas9 to chromatin modifiers such as p300 (activator), DNMT3A (repressor), or TET1 (demethylase), transcription of target genes can be activated or silenced without introducing double-strand breaks or altering the DNA sequence.

Together, these new tools are redefining the field of functional mutagenesis and providing plant scientists and breeders with a range of precision technologies that can be used for a variety of goals, from market-driven trait improvement to the discovery of gene functions. The next generation of resilient, high-performing, and sustainable crops will be greatly influenced by their ongoing development as well as advancements in genome annotation, regulatory frameworks, and delivery techniques.

Table 7: Emerging Innovations in Plant Genome Editing

Innovation	Description	Advantages	Examples
Base Editing	Direct conversion of one DNA base to another (e.g., C→T or A→G) without inducing double-strand breaks (DSBs)	High precision, minimal indels, fewer off-target effects	Editing the *ALS* gene in rice to confer herbicide tolerance
Prime Editing	Introduces targeted insertions, deletions, and base substitutions using a reverse transcriptase fused to	Broader editing range, versatile modifications, no	Proof-of-concept demonstrated in rice and wheat, but not yet widely

Innovation	Description	Advantages	Examples
	Cas9 nickase and a prime editing guide RNA	DSBs required	applied in breeding programs
Epigenome Editing	Modulates gene expression via targeted recruitment of chromatin modifiers (e.g., DNA methyltransferases, histone deacetylases) without altering DNA sequence	Reversible, non-transgenic (non-GMO), fine-tunes gene expression	Early-stage application in *Arabidopsis* and rice; potential for stress and yield trait regulation

7. Research Gaps

Although genome editing technologies have the potential to revolutionise society, there are still a number of significant research and implementation issues that need to be addressed. The sensitivity of off-target detection techniques is one significant drawback. Despite their widespread use, methods such as GUIDE-seq and Digenome-seq might not be able to identify all unwanted edits, especially in complex and polyploid plant genomes. Improved specificity is provided by high-fidelity Cas variants such as SpCas9-HF1 and eSpCas9, but off-target risks are still present (Zhang et al., 2015; Manghwar et al., 2019). Genetic redundancy and trait pleiotropy are major concerns as well. Numerous genes that are the focus of editing have pleiotropic effects or are members of multigene families, which can lead to unexpected phenotypic outcomes and make it more difficult to choose desirable traits. This emphasises the necessity of thorough multi-trait analysis and a more thorough comprehension of gene networks (Huang et al., 2021). The limited functional annotation of underutilised and non-model crops presents a third difficulty. The efficient use of genome editing in these species is hampered by the scarcity of high-quality reference genomes as well as a lack of understanding regarding regulatory elements and epistatic interactions (Michael & VanBuren, 2020). Additionally, there are major obstacles to the commercialisation of genome-edited crops due to regulatory ambiguity and international disparities. The global regulatory landscape is fragmented and unclear because some regions, such as the European Union, maintain strict GMO laws, while other countries, such as the United States and Argentina, have adopted relatively permissive frameworks that exempt certain genome-edited plants from GMO regulations (Schmidt et al., 2020). For genome editing technologies to be widely adopted and used responsibly in agriculture, these gaps must be filled.

8. Strategic Priorities for Future Research

Future studies must focus on a few crucial areas in order to fully realize the potential of induced mutagenesis in crop improvement and to further develop the field. The creation of pan-genomic and haplotype-aware editing platforms that take into consideration intra-species genomic diversity and haplotype structures is one important avenue. This method would improve genome editing's accuracy and suitability for genetically diverse populations. Combining machine learning and artificial intelligence (AI) to improve various aspects of genome editing is another exciting approach. In order to speed up trait discovery and validation, these technologies can greatly enhance guide RNA design, more accurately predict gene function, and lessen the possibility of off-target mutations. Additionally, to get past the present drawbacks of genotype-dependent transformation and extend genome editing to resistant and orphan crops, advances in in planta transformation technologies—in particular, non-

tissue culture–based delivery methods such as pollen magnetofection, nanoparticle-mediated delivery, or viral vectors—are crucial. Lastly, to evaluate the stability, fitness, and environmental impact of edited traits under actual field conditions, long-term ecological and phenotypic evaluations are required. The safe and sustainable use of genome-edited crops in agriculture will eventually be made possible by these studies, which will be essential for comprehending trait durability, unintended consequences, and regulatory compliance.

9. Conclusion

The traditional, random mutagenic methods of induced mutagenesis are giving way to highly targeted and programmable genome editing technologies. This development is a reflection of more general scientific breakthroughs in genomics, bioinformatics, and molecular biology that have enabled plant breeders to more accurately control genetic variation. While the foundation for genetic diversity has been established by conventional mutagenesis employing chemical and physical agents, the use of cutting-edge instruments such as CRISPR/Cas systems, base editors, prime editors, and doubled haploid technologies has greatly sped up and improved the accuracy of crop improvement. Breeding cycles can be shortened and trait stability improved by strategically modifying certain genes linked to stress tolerance, yield optimisation, and quality enhancement. However, current obstacles such as off-target effects, ineffective transformation systems in some crops, a lack of functional genomics data for non-model species, and disjointed regulatory frameworks must be addressed if modern mutagenesis is to reach its full potential. It will take a multidisciplinary strategy combining knowledge of plant biology, bioengineering, computational modelling, and regulatory science to overcome these constraints.

Furthermore, responsible genome editing implementation combined with thorough public involvement and long-term ecological assessments will be necessary for future advancement. To maintain public confidence and promote the adoption of genome-edited crops, open communication and unified international regulations will be essential. As these technologies advance, their prudent and scientifically informed use will be essential to securing food systems for an expanding world population, improving climate resilience, and attaining sustainable agricultural intensification.

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