Plant Archives Vol. 26, Supplement 1, 2026 pp. 3263-3279 e-ISSN:2581-6063 (online), ISSN:0972-5210

Plant Archives

Journal homepage: http://www.plantarchives.org
DOI Url : https://doi.org/10.51470/PLANTARCHIVES.2026.v26.supplement-1.436

GENOME EDITING TECHNOLOGIES IN PLANT BREEDING: SCIENTIFIC FOUNDATIONS AND BREEDING APPLICATIONS

Nishat Perveen[1], Ehsas Pachauri[2], Bhavani[3], Afsar Ahmad[4], Pankaj Kumar[5], Jagdeep Singh[6], Priyanka Kumari[7], Debolina Sinha[8], Ashwani Kumar Singh[9*] and Sachin Kashyap[10]

[1]Genetics and Plant breeding, Indira Gandhi Krishi Vishwavidyalaya, Raipur, C.G., India
[2]Department of Genetics and Plant Breeding, Indian Agricultural Research Institute, ICAR-CAZRI Jodhpur Hub, Rajasthan- 342003, India
[3]Department of Genetics and Plant Breeding, University of Agricultural Sciences, Dharwad, Karnataka, India
[4]Center of Agricultural Education, faculty of Agricultural Science, Aligarh Muslim University, Aligarh U.P., India
[5]Genetics and Plant Breeding, CoHA, Chirbatiya, camp at Ranichauri, VCSG UUHF, Tehri Garhwal 249199, Uttarakhand, India
[6]Department of Agriculture, Maharishi Markandeshwar University, Mullana, India
[7] Department of Agricultural Sciences, Radha Govind University, Ramgarh, Jharkhand, India
[8] Department of Agriculture, Netaji Subhas University, Jamshedpur, India
[9]Department of Genetics & Plant Breeding, Jaipur National University, Jaipur, Rajasthan, India
[10]ICAR - Sugarcane Breeding Institute, Regional Centre, Karnal, Haryana, India
*Corresponding author E-mail: agrimonu700@gmail.com
(Date of Receiving : 28-11-2025; Date of Acceptance : 29-01-2026)

ABSTRACT

In order to develop sustainable agriculture and food production in the world, the need has never been greater to improve our knowledge of how to modify plants so they can produce better yields in less time, under more extreme weather conditions, and be able to grow using fewer inputs. However, there are many obstacles to overcome before this can occur. One of these obstacles is the lack of genetic diversity among current crop varieties which limit our ability to select traits such as improved drought tolerance, higher yields and other desirable traits. In addition, the use of chemicals to control pests and diseases has resulted in many problems related to the development of resistant pest populations and increased chemical contamination of the environment. Finally, the rate at which new crop varieties are developed has slowed significantly since the introduction of the Green Revolution in the 1960's. As a result, researchers around the world have begun looking into ways to genetically modify plants to improve crop production. These genetic modifications could potentially address some or all of the issues described above (i.e., limited genetic diversity among crop varieties, pesticide/herbicide application, etc.). To achieve this goal, scientists are employing new technologies such as RNA interference (RNAi), gene silencing (knockdown) and most recently, genome editing technology (also known as gene editing). Of these three technologies, genome editing is the newest and arguably the most powerful tool available to date for modifying genes in plants and animals.

Keywords : Genome editing, CRISPR/Cas9, Plant breeding, Crop improvement, Base editing, Prime editing, Site-directed nucleases, Genetic transformation, Disease resistance, Abiotic stress tolerance, Regulatory framework, Food security

Introduction

Agriculture now faces numerous challenges as it strives to meet the world's demand for food for over 10 billion people expected by 2050, which will require increased food production of 25-70% to maintain current levels of food security. Additionally, the continued presence of climate change continues to create more severe biotic and abiotic stressors on crop productivity through such factors as drought, high temperature, soil salinity and the emergence of

additional pest and diseases (Ahmadikhah *et al*., 2025). As has always been the case with traditional plant breeding, this approach can be very time consuming and limited in terms of what can be achieved in terms of the amount of natural genetic diversity that exists in the gene pool of crops (Brock *et al*., 2025). Typically, the generation of new crop varieties using conventional breeding involves many generations of cross pollination and selection, which can take many years or even decades for certain perennial crops (Sahu *et al*., 2024). With the introduction of genetic engineering in the latter half of the 20th Century, the possibility of inserting foreign genes into crops was developed to produce genetically modified organisms. While transgenic approaches have had success with many crops, they have also encountered various regulatory barriers, public apprehension and concern about the random integration of foreign DNA into the genome of the plant (Waghmode *et al*., 2024). All of the above has generated a clear and present need for alternative methods of crop improvement that are faster, more efficient and more socially acceptable than those currently being used.Genome editing technologies have become a major breakthrough in addressing this need as they enable scientists to make targeted modifications to specific genomic loci in a manner that is much more precise than ever before (Gautam & Verma, 2025). Genome editing differs from previous mutagenesis techniques, where scientists were able to randomly introduce mutations across the entire genome, because genome editing enables scientists to intentionally modify the genome at the location(s) of choice (Ahmadikhah *et al*., 2025). Furthermore, compared to previous methods of crop improvement, genome editing reduces the time and resources needed to develop new crops (Ahmadikhah *et al*., 2025). Genome editing has evolved through successive generations of tools, beginning with early meganucleases and progressing to zinc finger nucleases, transcription activator-like effector nucleases, and finally the groundbreaking CRISPR/Cas systems (Tamizi *et al*., 2025). CRISPR/Cas9 systems have been recognized as among the greatest biotechnological breakthroughs of the 21st Century (Mitchell, 2025). CRISPR/Cas9 is the adapted form of the bacterial adaptive immune system that is a versatile, simple and efficient tool for making precise genome modifications in nearly all organisms, including plants (Waghmode *et al*., 2024). The ease of use of CRISPR/Cas9 systems, as demonstrated by the fact that scientists only need to design a single guide RNA to direct the Cas9 nuclease to the target site, has made genome editing widely accessible and has expedited its implementation in plant research and breeding programs around the globe (Gautam & Verma, 2025).

Evolution of Genome Editing Technologies in Plants

First-Generation Tools: Meganucleases

Second-Generation Tools: Zinc Finger Nucleases (ZFNs)

Beginning with Text Zinc Finger Nucleases were an important innovation within the area of Genome Editing Technology. ZFN's consist of two functional domains - a zinc finger DNA binding domain and a FokI endonuclease cleavage domain. The zinc finger DNA binding domain is used to bind to the target DNA sequence by recognizing a specific DNA sequence. The FokI endonuclease cleavage domain is responsible for cleaving the targeted DNA. With each zinc finger module able to recognize approximately three base pairs of DNA, ZFNs allow researchers to assemble multiple fingers to recognize much longer DNA sequences. As a result of the modular nature of ZFNs, researchers have been able to achieve greater flexibility in designing ZFNs to target very long, specific genomic loci compared to other technologies such as meganucleases. However, due to the complexity, cost, and time required to design and assemble ZFN pairs for each desired target locus, ZFNs have largely been inaccessible to less well-resourced laboratories. In addition, researchers using ZFNs have experienced problems related to the context dependent nature of DNA binding by ZFNs; resulting in decreased specificity and increased rates of off target effects (Keul *et al*., 2022).

Third-Generation Tools: Transcription Activator-Like Effector Nucleases (TALENs)

The transcription activator-like effector nucleases were developed to be superior to ZFNs through having greater predictability of their DNA binding (Brock *et al*., 2025). The TALENs are composed of customizable DNA-binding domains obtained from plant pathogens of Xanthomonas species fused with FokI nuclease domains (Ahmadikhah *et al*., 2025). Due to each TALE repeat module recognizing one base pair, the design of TALENs is much easier and more predictable than that of ZFNs (Tamizi *et al*., 2025). In addition, TALENs have been shown to be more specific and efficient than ZFNs and have been used successfully in many different plant species (Keul *et al*., 2022). Although TALENs have many positive attributes, the development of TALEN pairs has been time consuming and difficult due to the need to assemble many repeat modules to obtain a pair of TALENs that can bind to the same target site; also, the

large size of TALENs can make it difficult to deliver them into the cells of plants (Gautam & Verma, 2025).

Revolutionary Tool: CRISPR/Cas Systems

In addition to the application of CRISPR/Cas systems as a new type of adaptive immune response by bacteria against viruses (Waghmode *et al*., 2024), CRISPR/Cas systems were first applied to plant genome editing in 2012 and rapidly became the most widely used genome editing tool in plant biotechnology (Mitchell, 2025). CRISPR/Cas systems offer a number of significant advantages that have facilitated their global adoption; they are more efficient than previously available genome editing technologies, can be designed using only an easily generated guide RNA sequence, are inexpensive to use making them accessible to researchers around the world, can allow for simultaneous editing of many different genes through multiplexing, and are highly versatile and can be applied to a wide range of plant species (Gautam & Verma, 2025). There is now clear evidence of the comparative advantage of CRISPR/Cas systems over all previous genome editing tools. CRISPR/Cas systems allow for higher editing efficiencies, are much easier to design and implement than the alternative tools, are less expensive to use than the alternative tools, result in a significantly faster turnaround time than the alternative tools, and allow for more flexibility in terms of multiplexing than the alternative tools such as ZFNs and TALENs (Ahmadikhah *et al*., 2025). These advantages have resulted in a very rapid global adoption of CRISPR technology into plant breeding programs, and the applications of this technology are rapidly extending beyond basic research into commercially developed crops (Mitchell, 2025).

Scientific Foundations of CRISPR/Cas Systems

CRISPR/Cas9 Mechanism and Components

The CRISPR/Cas9 system has two essential elements; the Cas9 endonuclease enzyme, and a single guide RNA (sgRNA) (Waghmode *et al*., 2024). The sgRNA is a synthetic combination of two RNA strands: the CRISPR RNA (crRNA), which has about 20 nucleotides complementary to the target DNA sequence, and the trans-activating crRNA (tracrRNA) which binds with Cas9 (Gautam & Verma, 2025). The sgRNA guides Cas9 nuclease to a particular genomic position by base pairing between the guide sequence and the target DNA via Watson-Crick hydrogen bonds (Mitchell, 2025).

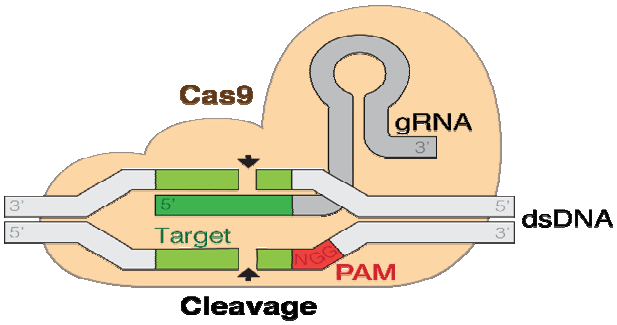

Fig. 1 : CRISPR/Cas9 Mechanism

In order for Cas9 to recognize the target, there needs to be a Protospacer Adjacent Motif, a short DNA sequence immediately next to the target site (Waghmode *et al*., 2024). For the Streptococcus pyogenes Cas9 (SpCas9), the most commonly used variant, the PAM sequence is NGG (Where N = any nucleotide) (Gautam & Verma, 2025). After the sgRNA-Cas9 complex binds to the target DNA and recognizes the PAM sequence, Cas9 creates a double strand break 3 base pairs upstream of the PAM sequence (Mitchell, 2025). Once the double strand breaks are created, the cell can use its native DNA repair machinery to fix the damage, primarily using one of two different repair mechanisms. Non-homologous End Joining (NHEJ) is an error prone repair mechanism that frequently creates small insertions or deletions at the break site resulting in frameshift mutations and gene knockout events (Waghmode *et al*., 2024). Homology Directed Repair (HDR) is a highly accurate repair mechanism that utilizes a template DNA molecule to correct the break allowing for the precise editing of genes including nucleotide substitutions, gene additions, and gene replacement as long as a suitable donor template is available (Gautam & Verma, 2025).

CRISPR/Cas Variants and Alternative Systems

While Cas9 is still the most commonly utilized CRISPR nuclease, other Cas proteins have been identified, isolated and investigated for potential use in plant genome editing (Gallo *et al*., 2025). The Cas12a (previously referred to as Cpf1) enzyme is produced by various bacteria and offers several unique characteristics compared to Cas9. First, Cas12a utilizes a crRNA only, does not require a tracrRNA; secondly, it identifies a T-rich PAM sequence (TTTN); thirdly, it produces staggered DNA ends rather than producing blunt DNA ends; and lastly, Cas12a has an inherent RNase activity which allows for cleavage of multiple guide RNAs from a single transcript (Gautam & Verma, 2025). Cas13 specifically targets RNA and provides the opportunity for RNA editing, gene

silencing and diagnostic uses without causing irreversible genome alterations (Gallo *et al*., 2025). In order to extend the capabilities and increase the precision of genome editing, numerous engineered Cas9 variants have been created. The Cas9 nickase (nCas9) is a variant of Cas9 that includes mutations which inactivate one of the two nuclease domains resulting in the formation of single-stranded breaks as opposed to double-stranded breaks (Gautam & Verma, 2025). The dead Cas9 (dCas9) variant is similar to nCas9 but dCas9 also has its nuclease domains inactivated; however, dCas9 retains the ability to bind to DNA and can be used for transcriptional regulation and epigenome editing if fused with activator or repressor domains (McLaughlin *et al*., 2025). Several high-fidelity Cas9 variants have also been engineered to reduce off-target activity and maintain on-target efficiency (Gallo *et al*., 2025).

Table 1: Comparison of Genome Editing Technologies for Plant Breeding

Technology	Mechanism	Advantages	Limitations	Applications in Plants
Meganucleases	Natural restriction enzymes with long recognition sites (12-40 bp)	High specificity; minimal off-targets	Difficult to engineer for new targets; limited flexibility	Limited use; proof of concept studies
ZFNs	Zinc finger DNA-binding domains fused to FokI nuclease	Modular design; programmable targeting	Complex design; context-dependent binding; expensive	Disease resistance; herbicide tolerance
TALENs	TALE repeats fused to FokI nuclease	Predictable DNA recognition; high specificity	Large size; laborious assembly	Disease resistance; quality traits
CRISPR/Cas9	RNA-guided Cas9 nuclease	Simple design; cost-effective; multiplexing; high efficiency	PAM requirement; potential off-targets	All major breeding applications
CRISPR/Cas12a	RNA-guided Cas12a nuclease	T-rich PAM; staggered cuts; RNase activity	Lower efficiency than Cas9 in some species	Multiplex editing; trait stacking
Base Editors	dCas9/nCas9 fused to deaminase	No DSBs; precise base conversion; no donor template needed	Limited editing window; bystander edits	Herbicide resistance; nutritional traits
Prime Editors	nCas9 fused to reverse transcriptase	All base conversions; insertions/deletions; no DSBs	Lower efficiency; complex design	Trait fine-tuning; SNP correction

(Gallo *et al*., 2025).

Base Editing Technology

In this paper we are going to show how base editing has moved precision genome editing forward as a tool that can directly convert one DNA base into another without generating a DSB (double-strand break) or needing to use a template of donor DNA (Li *et al*., 2023). The two most common types of base editors that have been developed are: cytosine (C) base editors, which convert C•G base pairs to T•A base pairs, using a cytidine deaminase enzyme fused to either a Cas9 nickase or a dead Cas9 (Li *et al*., 2022), and adenine (A) base editors, which convert A•T base pairs to G•C base pairs, using an adenine deaminase enzyme fused to various forms of Cas9 (Li *et al*., 2023).

The base editor system functions in a stepwise manner. First the Cas9 component is directed to bind to the target DNA sequence by an sgRNA; secondly the deaminase enzyme within the editing window of about 5 nucleotides (nucleic acids) of the target site changes the base being converted; thirdly, the cell's DNA repair machinery resolves the changed base and fourthly, the nicks in each of the two strands of the DNA are repaired so that the permanent base conversion is established (Li *et al*., 2022). The potential for base editing to be used in plant breeding includes the ability to make specific point mutations in plants without making DSBs (double-strand breaks), without needing to provide a template of donor DNA, lessening the amount of indels produced by conventional CRISPR/Cas9 and the ability to correct SNPs (single-nucleotide polymorphisms) associated with plant agronomic characteristics (Li *et al*., 2023). In crops base editing has been shown to create herbicide resistance through targeted mutations in acetolactate synthase genes, improve nutritional quality, increase disease resistance through modification of susceptibility genes and enhance abiotic stress tolerance (Nakazato *et al*., 2024).

Prime Editing Technology

The prime editor is a powerful "search-and-replace" genome editing tool capable of performing all twelve types of base-to-base changes, all insertion changes, and all deletion changes without causing double-strand breaks or using donor DNA (Tian *et al*., 2025). The prime editor is created by fusing a nicking Cas9 variant (H840A) to a modified version of the reverse transcription enzyme (RT), known as Li *et al.* (2022). Instead of utilizing a traditional sgRNA to locate the target site, prime editing utilizes a guide RNA referred to as a prime editing guide RNA (pegRNA), which contains two regions; the first is the target-binding region and the second is the region where the primer is attached to an RT template encoding the intended change (Vu *et al*., 2023). Once the prime editing process begins, the Cas9 nickase component will bind to the target site and create a single-stranded break on the non-targeted DNA strand, the RT component of the prime editing system will then utilize the 3' flap as a primer to synthesize new DNA from the RT template within the pegRNA creating a copy of the intended change into the genome, the newly synthesized DNA is then allowed to compete with the original non-edited DNA strand for incorporation and finally, the cell's DNA repair and replication machinery are able to resolve the edited and unedited DNA strands (Tian *et al*., 2025). To increase the efficiency of this process, a second sgRNA may be introduced to create a single-stranded break in the non-edited strand allowing it to be replaced by the edited strand (Li *et al*., 2022). One of the primary advantages of prime editing is the ability to make virtually any type of edit into the genome, including but not limited to, all transitions and transversions, targeted insertions ranging from a few nucleotides up to several dozen nucleotides, targeted deletions of varying size, and combinations of substitutions, insertions, and deletions (Vu *et al*., 2023). In addition to applications in animal breeding, prime editing could also be used in plant breeding to correct genetic mutations associated with desirable traits, introduce Single Nucleotide Polymorphisms (SNPs) from elite crop lines, modify regulatory sequences to modulate gene expression, and create entirely new alleles (Tian *et al*., 2025). While prime editing has the potential to revolutionize many areas of genetics research, there are still some major hurdles that need to be overcome before it can be utilized effectively in plants, such as the wide variability in editing efficiencies among different targets and species, reduced editing efficiencies in dicot plants relative to monocot plants, need for optimization of pegRNA design and expression, and need for reverse transcription variants with increased enzymatic activity (Vu *et al*., 2022).

Delivery Methods and Transformation Techniques

Agrobacterium-Mediated Transformation

Transformation using Agrobacterium continues to be one of the primary ways scientists deliver CRISPR/Cas to plant cells, particularly to dicots (Ebrahimi & Hashemi, 2024). As mentioned earlier, Agrobacterium uses the Ti plasmid system to naturally deliver DNA into plant cells (Mitchell, 2025), so researchers utilize a similar mechanism by cloning both the CRISPR/Cas9 cassette and guide RNA expression cassette into a binary vector that can be inserted into an Agrobacterium strain (Ebrahimi & Hashemi, 2024). Researchers then co-culture the transformed Agrobacterium with plant explants, resulting in the T-DNA of the edited components being delivered and incorporated into the plant genome (Kim *et al*., 2025). There are benefits to utilizing Agrobacterium mediated transformation, they include relative ease of transformation into various plant species, long-term stable integration allowing for constitutive expression of the edited components, well established transformation protocols for many crop species, and low cost when compared to other transformation methods (Ebrahimi & Hashemi, 2024). Nevertheless, there are also limitations to this transformation method, such as transformation efficiency depending on plant genotype and limiting transformation in recalcitrant varieties, the necessity for tissue culture and subsequent regeneration, a time-consuming process of transformation to generation of new plants, and the possibility of unwanted T-DNA integration (Kumar *et al*., 2025). There have been recent improvements made to increase the efficiency of genome editing via Agrobacterium mediated transformation. For example, the creation of ternary vector systems, each containing additional virulence helper plasmids, has greatly increased T-DNA delivery (Aliu *et al*., 2024). Auxotrophic Agrobacterium strains allow researchers to remove these bacteria after co-cultivation, thus minimizing contamination (Aliu *et al*., 2024). In planta transformation eliminates the need for tissue culture by utilizing techniques like floral dipping of Arabidopsis or injection of meristematic tissues (Toda *et al*., 2023). Finally, researchers must optimize strain selection as some Agrobacterium strains exhibit differing efficiencies of transformation within plant species (Kaba *et al*., 2025).

Biolistic Transformation (Gene Gun)

The biolistic transformation process commonly referred to as particle bombardment or "gene gun"

technology is a physical means of delivering genetic material that contains CRISPR components by accelerating coated microparticles at high pressures into plant cells (Kumar *et al*., 2025). The advantage of this technique lies in its capability to introduce DNA into virtually all plant cells or tissues regardless of species (Ebrahimi & Hashemi, 2024). Biolistic transformation offers unique benefits over other methods including: (1) the ability to successfully transform recalcitrant plant species and genotypes which have been difficult to transform using Agrobacterium; (2) the ability to directly deliver genetic material into chloroplast and mitochondrial genomes; (3) direct delivery of genetic material into intact tissues without needing to create protoplasts; (4) independence from plant-based host factors required for Agrobacterium-mediated transformation (Hoengenaert *et al*., 2023). However, several drawbacks exist in utilizing biolistic transformation including: (1) damage to the tissue due to particle bombardment; (2) increased likelihood of transgene insertion at random genomic loci; (3) variability in transformation efficiency that requires optimization of parameters; and (4) potential for multi-copy insertions (Salvagnin *et al*., 2023).

Ribonucleoprotein (RNP) Delivery

The assembly of pre-packaged CRISPR/Cas ribonucleoproteins represents an important step toward the achievement of transgene-free genome editing (Gong *et al*., 2021). RNPs are assembled by mixing purified Cas protein with in vitro-synthesized guide RNA (which is produced using standard molecular biology techniques) and introducing the mixture into plant cells (Mitchell, 2025). Therefore, this method eliminates the necessity for integrating foreign DNA into the genome of the target organism to encode the editing components. There are many critical benefits to RNP delivery for crop improvement. The first benefit is that transgene-free editing allows for the generation of genome-edited crops that do not contain integrated foreign DNA, and therefore may have fewer regulatory hurdles (Creeth *et al*., 2025). The second is that transient activity means that once the RNPs have completed their editing function they will degrade quickly, thus reducing the likelihood of off-target effects (Gong *et al*., 2021). The third benefit is that RNP delivery allows for immediate editing upon delivery, eliminating the time required for transcription and translation (Malnoy *et al*., 2016). Finally, the use of efficient screening methods reduces the necessity for selecting edited lines based on drug resistance (Zhang *et al*., 2022). Protoplast transfection is currently the most common method for delivering RNPs into plant cells; it involves removing the cell wall from a plant cell to produce a protoplast (Creeth *et al*., 2025). The RNPs are then introduced into the protoplast using either PEG-mediated uptake or electroporation (Gambino *et al*., 2024). Protoplasts containing edited genomes are then regenerated into whole plants through the use of optimized tissue culture protocols (Sahab *et al*., 2024). Protoplast-based RNP delivery has resulted in very high editing efficiencies in a variety of plant species including rice, citrus, grapevine, apple, and canola (Malnoy *et al*., 2016).

Viral Vector-Based Delivery

Plant viruses have been engineered to serve as vectors for delivering CRISPR components to plant cells, allowing for a tissue culture free approach to genome editing (Uranga & Dars, 2022). Virus-induced genome editing relies on the inherent ability of plant viruses to replicate and to move systemically through plants (Han *et al*., 2025). A number of plant viruses have been adapted to deliver guide RNAs (gRNAs) or entire CRISPR cassettes, including tobacco rattle virus, potato virus X, and several types of geminiviruses (Uranga & Dars, 2022). The delivery of CRISPR components by viruses provides several unique advantages over other delivery methods: no requirement for tissue culture, the ability to edit multiple parts of the plant due to systemic movement of the virus, rapid editing without the need for stable transformation, and possible field application through simple inoculation methods (Han *et al*., 2025). However, there are also a number of challenges associated with using viruses to deliver CRISPR components: limited cargo capacity which restricts the size of the gene that can be delivered, potential triggering of plant immune response to the presence of the virus, genome size constraints of the viral vector, and limited host range of most plant viruses (Uranga & Dars, 2022).

Nanoparticle-Mediated Delivery

The advantages of nanoparticle delivery include:

- Protection of cargo from degradation,
- Tunability of physical and chemical properties to achieve the best possible cell uptake,
- Targeted delivery to certain organs, and
- Less dependence on tissue culture in some cases (Qin *et al*., 2025).

Applications are currently being developed for spray delivery onto leaves, for the direct infiltration of nanoparticles into plant tissues, and in combination with other transformation methods to increase efficiency (Shivashakarappa *et al*., 2025). Han *et al*.

(2025) identified that nanoparticles offer an attractive method to deliver CRISPR components to plant cells. Shivashankarappa *et al*. (2025) described how various types of nanoparticles such as mesoporous silica nanoparticles, carbon nanotubes, and functionalized graphene oxide have been used to develop gene-delivery systems. Nanoparticles have the ability to carry both proteins, RNA, and DNA as their cargo, and they can be designed to either passively or actively enter cells (Shivashankarappa *et al*., 2025).

Applications in Plant Breeding

Disease Resistance

Genome editing has made it possible to develop genetically modified disease-resistant crops. The development of disease-resistant crops has been possible through the creation of genetic modifications of susceptibility genes that pathogens infect to cause disease and the development of new resistance mechanisms (Bhalerao *et al*., 2025). The two main strategies to create disease-resistant crops are: editing of susceptibility genes which are used by pathogens to infect the host and the creation of new or improved resistance genes or pathways to provide additional protection to the host (Manzoor *et al*., 2024). Editing of susceptibility genes creates an environment where the plant becomes resistant to the pathogen but does not disrupt normal growth and development of the plant (Faizal *et al*., 2024). Examples of susceptibility gene editing include: the disruption of MLO genes to confer broad-spectrum resistance to powdery mildew in tomato, wheat, and grapevine (Malnoy *et al*., 2016); the modification of eIF4E genes to confer resistance to potyviruses in many different types of crops (Mushtaq *et al*., 2019) and; the editing of SWEET genes to prevent the bacterial blight pathogen from infecting rice (Bhalerao *et al*., 2025).

Enhancing resistance mechanisms of plants through genome editing can be done through various methods including the editing of genes involved in the pattern recognition receptor pathway, strengthening of the signal transduction pathway and the accumulation of antimicrobial compounds (McLaughlin *et al*., 2025). CRISPR activation has been used to activate defense-related genes as shown by the activation of SlPAL2 to increase the resistance to bacterial canker in tomato (Rivera-Toro *et al*., 2025). There is also research on the direct editing of pathogen genomes using CRISPR to improve resistance to viruses, which includes the design of guide RNAs to cut viral DNA or RNA (Jahan *et al*., 2025).

Abiotic stress tolerance

Abbiotic stresses are one of the most important environmental stresses affecting crop yields worldwide. These abiotic stresses include drought, heat, salinity and cold. The intensification of abiotic stresses due to climate change has increased the importance of developing crop varieties with abiotic stress tolerance (Mann *et al*., 2024). Genome editing provides a method to specifically modify genes and regulatory elements controlling abiotic stress response in crops (Chavhan *et al*., 2025). Drought tolerance has been improved through genome editing by the modification of genes regulating stomatal closure and water use efficiency, modification of ABA signaling pathways and modification of root architecture for deeper water access (Kumar *et al*., 2023). Genome editing has also improved heat tolerance by modifying heat shock factors and heat shock proteins, modifying the membrane stability at high temperature, and improving the photosynthetic efficiency at high temperature (Chavhan *et al*., 2025). Saline soil affects a large portion of land globally. Salt tolerance has been improved through genome editing of sodium transporters to decrease the amount of Na+ in the shoot tissue of the plant, increasing the amount of sodium ions that are stored in the vacuole, and modifying the osmotic adjustment mechanism (Wang *et al*., 2022). Cold tolerance has been improved through genome editing of C-repeat binding factor and cold responsive genes, modifying the fluidity of membranes at low temperature, and increasing the production of protective metabolites (Erdoan *et al*., 2023). Heavy metal toxicity is another abiotic stress that can affect the growth and development of crops. Genome editing has been used to improve heavy metal tolerance by editing metal transporters and chelators, increasing the ability to sequester metals into cellular compartments, and modifying the detoxification pathways (Kumar *et al*., 2023).

Yield Improvement

Yield of grains is the major goal of crop improvement, and it is a complex quantitative trait determined by multiple genes (Niraula *et al*., 2024). Genome editing allows for the precise modification of yield components such as grain number, grain size, grain weight, tillering and branching, and harvest index (Achary & Reddy, 2021). Modification of the genes controlling grain size such as GW2, GS3 and GS5 in rice has created varieties with larger grain sizes (Achary & Reddy, 2021). Editing of genes controlling the number of spikes per panicle has increased the number of spikes per panicle (Zeb *et al*., 2022). Optimization of plant architecture through genome

editing of genes controlling plant height has created semi-dwarf varieties with improved lodging resistance (Chen, 2024). Modification of genes controlling tillering has improved the number of productive tillers (Zeb *et al*., 2022). Improvement of photosynthesis efficiency involves genome editing to increase carbon fixation efficiency, increase chlorophyll content and increase light use efficiency (Niraula *et al*., 2024). Extending the grain filling period through delayed senescence has been achieved by editing senescence-associated genes (Zeb *et al*., 2022).

Nutritional Quality Enhancement

Improving the nutrition level of staple crops is important for reducing malnutrition and "hidden hunger" that affect millions around the world (Tadkal *et al*., 2024). Biofortification with genome editing allows for the specific alteration of biosynthetic pathways and nutrient transporters (Niraula *et al*., 2024). For example, mineral enrichment can be accomplished by editing genes encoding metal transporters and/or storage proteins to increase the concentration of minerals such as iron, zinc, calcium, etc. (Achary & Reddy, 2021). Editing genes involved in the synthesis of anti-nutrients (e.g., phytic acid) also increases the bioavailability of minerals (Ukhatova *et al*., 2023). The enhancement of vitamins include modifications to carotenoid biosynthesis pathways to increase the content of provitamin A, increased tocopherol (vitamin E) content, improved folate and other B-vitamin levels, etc. (Niraula *et al*., 2024). The improvement of protein quality includes modifications to the amino acid content to improve the essential amino acid content, reductions in the amount of allergenic proteins and improvements in protein digestibility (Tadkal *et al*., 2024). Finally, the modification of oil composition in oil seed crops includes reducing the content of saturated fatty acids to produce healthier oils, increasing oleic acid content, and incorporating omega-3 fatty acids into the crop (T. *et al*., 2025).

Quality Traits and Post-Harvest Characteristics

Horticulture crops are genetically edited to improve quality and shelf life. The quality improvements can be to increase the sugar content or flavor compounds in fruit, modify organic acids to achieve a perfect balance in terms of taste and increase the firmness and texture of fruits (Daniel *et al*., 2023). The extension of the shelf-life has been achieved by suppressing ethylene biosynthesis to delay the ripening process, reducing the activity of polyphenol oxidase to prevent browning, and increasing the integrity of the cell walls (Sahu *et al*., 2024). The color is modified by genetic editing of the anthocyanin biosynthesis pathway for purple-blue colors, carotenoids for yellow orange colors and chlorophyll degradation for green and red colors (Nonaka & Ezura, 2024). The quality of processing can also be modified by altering the starch composition of the processed crop for better suitability to a particular application, reducing the amount of acrylamide precursors in processing crops and improving the extraction efficiency (Karanam *et al*., 2024). Genetic editing can enable herbicide tolerance to develop by making targeted mutations to genes such as ALS and EPSPS. This will allow farmers to have an effective tool to manage weeds on their fields (Veillet *et al*., 2019).

Case Studies in Major Crops

Rice (*Oryza sativa*)

Zegeye *et al*. (2022) have identified that, due to its widespread use by more than 50% of the world's population, rice is an important crop to focus on in terms of genome editing research.

Zeb *et al*. (2022) have reported on successful uses of CRISPR/Cas9 to produce several different types of genetic modifications in rice; including both the yield of the rice plant and other characteristics of the rice plant such as plant structure, size and growth. Studies using CRISPR/Cas9 to modify the GW2 gene of rice found increased grain diameter, grain weight and increased nutritional value of the modified grains (Achary & Reddy, 2021). Genetic modifications using CRISPR/Cas9 to modify the IPA1 gene of rice produced plants having an increased grain number per plant and also improved the overall plant architecture (Zeb *et al*., 2022). CRISPR/Cas9 mediated genetic modification of the DEP1 gene in rice resulted in better developed panicles and increased grain number (Zeb *et al*., 2022). In addition to improving yield, CRISPR/Cas9-mediated genetic modification of the OsSWEET genes eliminated their function in rice, which conferred resistance to bacterial blight, while the disruption of eIF4G conferred resistance to rice tungro virus, and modifying the Pi21 gene increased the plant's ability to resist the fungal infection known as blast disease (Tripathi *et al*., 2022). Additionally, the modification of the OsPYL genes using CRISPR/Cas9 conferred drought tolerance and improved yields when grown under conditions of water deficit (U. Kumar *et al*., 2025). Modification of the OsNAC genes using CRISPR/Cas9 increased salt tolerance and drought tolerance in rice (Zeb *et al*., 2022). Finally, CRISPR/Cas9 was used to increase iron and zinc levels in rice and to lower phytic acid levels in rice, thereby increasing the availability of minerals from the diet

(Achary & Reddy, 2021), as well as to increase the amount of resistant starch in rice (Achary & Reddy, 2021).

Wheat (*Triticum aestivum*)

Wheat, being one of the main crops that is used by many people around the world and having a very large allohexaploid genome, creates both new opportunities and difficulties in the use of genome editing technology (Brock *et al*., 2025). The potential of CRISPR/Cas9 to edit homoeologous gene copies at the same time allows it to be an important tool in improving wheat (Shrawat & Armstrong, 2018). Examples of disease resistant traits created using genome editing technology include the simultaneous editing of all three MLO homoeologs which provides powdery mildew resistant wheat, modification of susceptibility genes to provide Fusarium head blight resistant wheat, and modification of stripe rust resistant wheat (Sampath *et al*., 2023).

Examples of quality traits improved through genome editing include lower gluten protein levels to develop low-gluten wheat varieties, modification of genes related to starch to alter starch properties, and improvement of dough quality characteristics (Shrawat & Armstrong, 2018). Genome editing has also been successfully applied to improve abiotic stress tolerance. These examples include improved drought tolerance, heat stress tolerance for adapting to changing climates, and salt tolerance for cultivating on marginal lands (Niraula *et al*., 2024).

Table 2: Major Applications of Genome Editing in Crop Improvement

Application Category	Target Traits	Genes/Pathways Edited	Crop Examples	Expected Benefits
Disease Resistance	Bacterial, fungal, viral resistance	MLO, SWEET, eIF4E, DMR6, S genes	Rice, wheat, tomato, citrus, banana	Reduced pesticide use; stable resistance
Abiotic Stress Tolerance	Drought, heat, salt, cold tolerance	DREB, HSP, SOS, NHX, ERECTA	Rice, wheat, maize, soybean	Climate resilience; expanded cultivation areas
Yield Enhancement	Grain size, number, weight; architecture	GW2, GS3, IPA1, DEP1, GIF1	Rice, wheat, maize, sorghum	Increased productivity; food security
Nutritional Quality	Vitamins, minerals, proteins, oils	Carotenoid pathway, metal transporters, fatty acid genes	Rice, wheat, maize, cassava, soybean	Improved human nutrition; biofortification
Quality Traits	Flavor, texture, shelf life, processing	Ethylene biosynthesis, polyphenol oxidase, amylose content	Tomato, potato, apple, banana	Consumer acceptance; reduced waste
Herbicide Tolerance	Resistance to specific herbicides	ALS, EPSPS, HPPD	Rice, maize, canola, soybean	Weed management; sustainable agriculture

(Tripathi *et al*., 2022)

Maize (*Zea mays*)

Maize is one of the world's most critical crops for food, feed and industrial purposes (Namata *et al*., 2025). The application of genome editing in maize has also benefitted from the development of more efficient transformation systems and new morphogenetic regulators (Azanu *et al*., 2024). There have been agronomic improvements through the genetic modification of plant architectural genes for optimal yields; enhancements of nitrogen use efficiency for more sustainable production; and improvements of genes related to drought tolerance (T. *et al*., 2025).

The development of disease and pest resistant maize cultivars involves editing for resistance to northern corn leaf blight; enhancing resistance to insect pests; and developing maize lethal necrosis resistant cultivars (Tripathi *et al*., 2022). Nutritionally, there have been enhancements through biofortification, increasing the provitamin A content of maize; improving the quality of proteins in maize by modifying amino acids; and improving the quality of oils in maize (Niraula *et al*., 2024). Additionally, there have been improvements in the quality of maize products through modifications in starch composition for industrial uses; reductions in anti-nutritional compounds; and improving the digestibility of the product (Karanam *et al*., 2024).

Tomato (*Solanum lycopersicum*)

Tomatoes serve as one of the world's leading vegetable crops and as a model organism for the study of fleshy fruit development (Sahu *et al*., 2024). Genome editing using CRISPR/Cas9 has been widely used for tomato improvement with many successful examples (Sahu *et al*., 2024). Fruit quality

improvements of tomatoes include increasing the lycopene and other carotenoids within the fruit; modifying the sugar/acid ratio to improve the taste of the fruit; and improving the texture and firmness of the fruit (Daniel *et al*., 2023). Extended shelf life of tomatoes has been developed through modifying the genes that contribute to ethylene biosynthesis, reducing the expression of the enzymes responsible for fruit softening, and delaying the ripening process (Sahu *et al*., 2024). Disease resistance improvements in tomatoes include knocking out the DMR6 gene which results in broad-spectrum disease resistance; modifying the genes responsible for susceptibility to bacterial speck disease; and improving the virus resistance of tomatoes (Manzoor *et al*., 2024). Finally, stress tolerance enhancements in tomatoes include improved salt tolerance; drought stress tolerance; and adaptation to heat stress (Erdoan *et al*., 2023).

Banana (*Musa* spp.)

Bananas and plantains are major staple crops in tropical regions, however, traditional breeding methods are limited due to sterility and long generation times (Tripathi & Tripathi, 2024). Genome editing may provide a faster method of improvement than traditional breeding (Tripathi & Tripathi, 2024). Disease resistance is a main goal for the development of genetically modified bananas, with the development of Fusarium wilt resistance through several methods; improvement of resistance to black sigatoka; and development of banana bunchy top virus resistance (Tripathi *et al*., 2022). Nutritional enhancement includes biofortification of bananas to increase the provitamin A content and enhance the levels of micronutrients (Tripathi & Tripathi, 2024).

Regulatory Landscape and Policy Framework

Global Regulatory Approaches

Regulatory treatment of genome-edited crops varies widely across countries and regions - creating a complex environment for developers and researchers (Sprink *et al*., 2022). There are two primary regulatory philosophies in place today: process-based regulation focuses on how the product was created; while product-based regulation focuses on characteristics of the final product (Sprink *et al*., 2016). The U.S. Has adopted a relatively permissive product-based approach (Wolt & Wolf, 2018). The USDA does not regulate genome-edited plants that could have been developed through traditional breeding or do not contain foreign DNA (Smyth, 2019). This regulatory stance has facilitated rapid development and commercialization of genome-edited crops in the U.S. Market (Wolt & Wolf, 2018). A number of genome-edited crops have been deregulated, including waxy corn, herbicide-tolerant canola, high-oleic soybean and mushrooms with reduced browning (Mamrutha *et al*., 2023).

European Union Regulatory Framework

The E.U. has taken a more restrictive approach following the 2018 ruling by the European Court of Justice (Ruffell, 2018). The court determined that organisms obtained through genome editing are subject to the GMO directive 2001/18/EC (Eckerstorfer *et al*., 2021). Therefore, genome-edited crops must undergo comprehensive risk assessments and authorization procedures (Ruffell, 2018). The ruling has generated significant controversy and calls for regulatory reform from the scientific community (Hundleby and Harwood, 2018). Implications of the E.U. Decision include mandatory labeling of genome-edited products, extensive safety and environmental assessments, lengthy approval processes potentially spanning years and restrictions on cultivation in E.U. Member states (Ruffell, 2018). Recent legislative developments indicate potential reform, with the European commission proposing new regulations for "plants obtained by certain new genomic techniques" (Juszczuk-Kubiak *et al*., 2025). The proposed framework would create categories based on nature of modifications, with some genome-edited plants potentially exempt from GMO regulations (Juszczuk-Kubiak *et al*., 2025).

Regulatory Approaches In Other Regions

Many countries have developed their own regulatory frameworks reflecting diverse approaches (Smyth, 2019). Argentina pioneered a trait-based regulatory system where products are evaluated based on whether they introduce novel traits or combinations (Whelan *et al*., 2020). This approach has enabled efficient review and approval of genome-edited crops (Whelan *et al*., 2020). Brazil has adopted a product-based approach similar to the United States, with genome-edited plants without foreign DNA exempt from GMO regulation (Smyth, 2019). Canada employes a product-based system focused on the novelty of traits rather than the method of development (Sprink *et al*., 2022). Australia has exempted sdn-1 genome editing (producing mutations equivalent to natural variations) from GMO regulation (Zhang *et al*., 2021). Japan does not regulate genome-edited organisms if they do not contain foreign DNA and could have arisen naturally (Smyth, 2019). China requires case-by-case evaluation with distinct categories for different types of genome editing (Smyth, 2019).

African Regulatory Developments

Several African countries have made significant progress in establishing enabling regulatory frameworks for genome editing (Tripathi *et al*., 2022). Nigeria published comprehensive national biosafety regulations for genome editing, becoming one of the first African countries to provide clear guidance (Tripathi *et al*., 2022). Kenya has developed regulatory guidelines for genome-edited crops and field testing (Tripathi *et al*., 2022). These developments are particularly significant given Africa's need for climate-resistant, disease-resistant crops (Amoah *et al*., 2024).

Social and Ethical Considerations

In addition to formal regulations, social acceptance and ethical considerations play important roles in the adoption of genome-edited crops (Ahmad *et al*., 2021). Public perception varies widely across regions, influenced by trust in regulatory institutions, previous experiences with GMOs, cultural attitudes toward food and agriculture, and media coverage and communication strategies (Hao *et al*., 2024). Transparency in the development and regulatory process is essential for building public trust (Waghmode *et al*., 2024). Clear communication of benefits and potential risks, engagement with stakeholders including farmers and consumers, and differentiation from traditional GMOs are important considerations (Lassoued *et al*., 2019).

Ethical considerations include equitable access to genome editing technology, avoiding excessive concentration of technology in developed countries, protection of farmers' rights and traditional knowledge, and consideration of biodiversity and environmental impacts (Ahmad *et al*., 2021). The regulatory landscape continues to evolve as countries gain experience with genome-edited crops and accumulate evidence regarding their safety and benefits (Sprink *et al*., 2022).

Challenges and Limitations

Off-Target Effects and Specificity

Although there are many highly specific CRISPR/Cas systems, off-target effects continue to be an issue (Son & Park, 2022). Off-target effects result from a Cas nuclease cleaving DNA at sites with partial complementarity to the guide RNA sequence (Erdoan *et al*., 2023). Genome-wide sequencing studies have demonstrated that CRISPR/Cas9 induced off-target mutations are relatively rare and often comparable to natural mutation rates; however, off-target effects should be minimized to achieve regulatory approval and to facilitate product development (Zegeye *et al*., 2022). Several strategies to minimize off-target effects include: (a) the utilization of high-fidelity Cas9 variants designed to enhance specificity, (b) the use of computational design of guide RNA sequences to avoid sequences with similar targets, (c) truncated guide RNAs to reduce tolerance for mismatches, and (d) paired nickase strategies that utilize two guide RNAs for cleavage (Erdoan *et al*., 2023). A comprehensive evaluation of off-target detection can involve (a) whole-genome sequencing of edited lines, (b) targeted deep sequencing of predicted off-target sites, and (c) unbiased genome-wide off-target detection assays (Modrzejewski *et al*., 2018).

Delivery Efficiency and Genotype Dependency

Delivery of editing components into plant cells and subsequent regeneration of edited plants represent significant hurdles (Han *et al*., 2025). Many elite crop varieties are recalcitrant to transformation due to their dependence on traditional transformation methods (Kumar *et al*., 2025). Several difficulties exist in achieving efficient transformation of important crop species and varieties, including lengthy tissue culture and regeneration times, somaclonal variation during tissue culture, and difficulty in obtaining homozygous edited lines (Gong *et al*., 2021).

Recent innovations to address these issues include the development of morphogenic regulators to increase transformation efficiency across genotypes, the development of in planta transformation techniques to eliminate the need for tissue culture, the development of genotype-independent transformation protocols, and improvements to protoplast regeneration systems (Han *et al*., 2025). Utilization of developmental regulators such as BABY BOOM and WUSCHEL have provided promising results to enhance transformation and regeneration efficiency in previously recalcitrant species (Ma *et al*., 2025).

Mosaicism and Editing Efficiency

Mosaicism, which includes chimeric or mosaic plants composed of edited and unedited cells, commonly occurs especially with transient delivery methods or when editing events occur late in the plant's life cycle (Butler *et al*., 2015). Mosaicism complicates the identification of uniformly edited plants and requires additional generations to obtain stably homogenous edited lines (Odipio *et al*., 2017). Several variables contribute to variable efficiencies of editing including chromatin accessibility at the target site, design and expression of guide RNA, stability and expression of Cas protein, the sequence and structure of the target site, and the type of plant cell and developmental stage of the plant (Tian *et al*., 2025).

Challenges Specific to Advanced Editing Tools

Base editors face several limitations, including limited editing windows of approximately 5 nucleotides, PAM sequence requirements limiting available target sites, potential for unwanted bystander edits within the editing window, and reduced efficiency in certain sequence contexts (Li *et al*., 2023). Prime editors also face challenges including significantly reduced editing efficiency relative to standard CRISPR/Cas9, complex pegRNA design and optimization, reduced efficiency in different plant species and target sites, and significantly reduced efficiency in dicots (Vu *et al*., 2022). Strategies to optimize base and prime editing include utilizing variants of reverse transcriptases with enhanced activity, designing pegRNAs that incorporate structural features, employing dual pegRNA strategies to enhance efficiency, and selecting systems to enrich for edited cells (Tian *et al*., 2025).

Intellectual Property and Access Issues

The current intellectual property environment for genome editing technologies is complicated by numerous patents that cover different aspects of the technology (Tripathi *et al*., 2023). Major concerns include concentrated patent ownership that limits access for public researchers and small businesses, licensing requirements that could limit access for developing countries, difficulties with "freedom to operate" for commercial developers, and costs to access patented technologies (Kalaitzandonakes *et al*., 2022). Some initiatives to provide greater access to genome editing technologies include patent pools and licensing arrangements, open source approaches for non-commercial research, humanitarian license agreements for developing countries, and the development of alternative editing platforms with clear intellectual property status (Tripathi *et al*., 2023).

Future Perspectives and Emerging Technologies

Integration with Multi-Omics and Systems Biology

The integration of genome editing with multi-omic analyses will enhance the process of crop improvement (Gautam & Verma, 2025). Genome editing, in conjunction with next-generation sequencing, allows for the identification of the best possible editing targets and the predictions of editing outcome (Chen, 2024). Using transcriptomics through RNA sequencing can help identify the regulatory elements that are most appropriate for the expression of genes as well as the off-target transcriptional effect caused by editing (Srivastava, 2025). Proteomics allows for the verification of the functional consequences of editing as well as the identification of compensatory mechanisms (Niraula *et al*., 2024). Using metabolomics, the modification of biochemical pathways and the nutritional value of an organism can be assessed (Tadkal *et al*., 2024). High-throughput phenomics can quickly characterize edited lines under various conditions (Chen, 2024).

Multiplexing and Trait Stacking

A key advantage of genome editing is its ability to modify several genes at once and thereby address polygenic traits (Abdelrahman *et al*., 2021). Strategies for multiplexing include the simultaneous editing of family members of a gene, the simultaneous modification of the multiple components of a biosynthetic pathway, the stacking of mechanisms of resistance for long-term disease resistance, and the pyramiding of traits for climate resilience (U. Kumar *et al*., 2025). Advanced multiplexing approaches utilize polycistronic guide RNA arrays for the expression of multiple guide RNAs from one construct, Csy4 processing for the liberation of individual guide RNAs, tRNA processing systems for guide RNA release, and pooled guide RNA libraries for forward genetic screens (Kumar *et al*., 2025).

Epigenome Editing and Gene Regulation

In addition to permanent alterations in the DNA sequence, epigenome editing offers the possibility of reversible regulation of gene expression without modifying the underlying sequence (McLaughlin *et al*., 2025). Applications include CRISPR activation for increasing the expression of beneficial genes RISPR interference for silencing a gene without modifying the sequence, targeted DNA methylation for heritable gene silencing, and histone modification for fine tuning the expression of genes (McLaughlin *et al*., 2025). These methods allow for dynamic control over gene expression patterns, which allows for the optimization of traits under various environmental conditions (Valencia-Lozano *et al*., 2024).

Speed Breeding and Accelerated Crop Improvement

Combining genome editing with speed breeding methods presents a synergistic method for significantly reducing the amount of time it takes to develop varieties (M. Ahmad, 2023). Speed breeding utilizes controlled environments including prolonged photoperiods, optimized temperature regimens, and early flowering induction to accelerate the rate of generation turnover (Sheri *et al*., 2025). When used in conjunction with genome editing, speed breeding can enable fast cycling from editing to phenotypic evaluation, fast development of homozygous lines via selfing or backcrossing, fast introgression of desirable

traits into elite backgrounds, and shortening of breeding cycles from decades to years (Fiaz, Wang, *et al*., 2021).

De Novo Domestication

One of the most ambitious uses of genome editing is the de novo domestication of wild species (Farinati *et al*., 2023). Instead of introgressing desirable traits from wild relatives into domesticated crops, this approach edits wild species to rapidly introduce domestication traits (Pak & Li, 2022). Examples of target modifications include reducing seed shattering and dormancy, increasing seed or fruit size, modifying plant architecture for mechanical harvesting, and eliminating anti-nutritional compounds (Farinati *et al*., 2023). This strategy may provide access to nutritionally superior or stress tolerant wild species for agriculture (Pak & Li, 2022).

Synthetic Biology Integration

Synthetic biology and genome editing together open new possibilities for improving crops (Sharma *et al*., 2022). Some examples of potential applications include the engineering of new biosynthetic pathways to produce pharmaceuticals or industrial products, the creation of synthetic gene networks to regulate complex traits, the development of biosensors to monitor in real time plant stress responses, and the design of synthetic chromosomes with improved gene arrangements (Huang & Liu, 2023). These types of applications go beyond the goals of traditional breeding programs and allow for the creation of plants with entirely new functions (Sharma *et al*., 2022).

Conclusion

Genome Editing Technologies (CRISPR/Cas) have revolutionized plant breeding by allowing for the most efficient, precise modification of genes at a particular locus within a genome. The transition from older "site directed" nucleases to newer versions like "base editors," "prime editors", etc., has dramatically accelerated the pace of technology development in this area. Additionally, there are a number of key differences between genome editing and both traditional plant breeding and conventional genetic engineering. Genome edited plants can be developed faster than those produced through traditional breeding; they can be developed with greater precision; they do not suffer from linkage drag; and they can be developed without transgenes, which could potentially provide less regulatory risk for growers in some countries.

The successful application of genome editing across all of the world's major crops has resulted in numerous examples of disease resistance improvements; abiotic stress tolerance enhancements; increased crop yields; and improved nutritional value. Examples include rice, wheat, corn, tomatoes and other crops where the use of genome editing has had a profound effect on the world's ability to address the agricultural challenges that it faces today. The ability to edit genomes simultaneously and in multiple places allows for the simultaneous improvement of multiple traits in one crop variety, which is important since most agronomic traits are controlled by multiple genes.

Although significant advancements have been made in the development and utilization of genome editing technologies, several challenges still exist. In addition to technical limitations (e.g., variability in gene editing efficiencies; off-target effects; delivery constraints; and genotype dependency), the global regulatory environment regarding genetically modified organisms remains fragmented and varies greatly among regions and jurisdictions, causing uncertainty for companies seeking to develop and export genetically modified crops. Additionally, public perception and societal concerns surrounding the development and consumption of genetically modified foods also play an important role in the rate of adoption of these products, and thus, it is critical to communicate the benefits of these products clearly and engage with stakeholders who have concerns about them.

In the future, the integration of genome editing with other technologies (e.g., multi-omics; speed breeding; artificial intelligence; and synthetic biology) will likely result in even more rapid advances in the development of new crop varieties. Newer approaches, including epigenome editing and de novo domestication, also represent exciting areas of research for plant breeders. As climate change increases in intensity and the global population grows, genome editing technologies will become increasingly important in the development of crop varieties that are resilient; productive; and nutritious -- and ultimately contribute to global food security. The continued refinement of these technologies, combined with science-based regulatory frameworks and the responsible deployment of these technologies, position genome editing as an indispensable tool for sustainable agricultural intensification in the 21st century and beyond.

References

Abdelrahman, M., Wei, Z., Rohila, J. S., & Zhao, K. (2021). Multiplex genome-editing technologies for revolutionizing plant biology and crop improvement. *Frontiers in Plant Science*, *12*, 721203.

Achary, V. M. M., & Reddy, M. K. (2021). CRISPR-Cas9 mediated mutation in GRAIN WIDTH and WEIGHT2 (GW2) locus improves aleurone layer and grain nutritional quality in rice. *Scientific Reports*, *11*(1), 21941.

Ahmad, A., Munawar, N., Khan, Z., Qusmani, A. T., Khan, S. H., Jamil, A., ... & Qari, S. H. (2021). An outlook on global regulatory landscape for genome-edited crops. *International journal of molecular sciences*, *22*(21), 11753.

Ahmad, M. (2023). Plant breeding advancements with "CRISPR-Cas" genome editing technologies will assist future food security. *Frontiers in plant science*, *14*, 1133036.

Ahmadikhah, A., Zarabizadeh, H., Nayeri, S., & Abbasi, M. S. (2025). Advancements in genome editing tools for genetic studies and crop improvement. *Frontiers in Plant Science*, *15*, 1370675.

Aliu, E., Ji, Q., Wlazlo, A., Grosic, S., Azanu, M. K., Wang, K., & Lee, K. (2024). Enhancing Agrobacterium-mediated plant transformation efficiency through improved ternary vector systems and auxotrophic strains. *Frontiers in Plant Science*, *15*, 1429353.

Amoah, P., Oumarou Mahamane, A. R., Byiringiro, M. H., Mahula, N. J., Manneh, N., Oluwasegun, Y. R., ... & Olasanmi, B. (2024). Genome editing in Sub-Saharan Africa: a game-changing strategy for climate change mitigation and sustainable agriculture. *GM Crops & Food*, *15*(1), 279-302.

Azanu, M. K., Kang, M., Lee, K., & Wang, K. (2025). Recent advances in Agrobacterium-mediated maize genetic transformation using immature embryos and seedling leaf whorl explants. *Cold Spring Harbor Protocols*, *2025*(11), pdb-top108460.

Bhalerao, S., Okoń, S., Rai, M., Jadhav, P., & Prokisch, J. (2025). Recent Advances in the CRISPR/Cas9 Technology for Engineering Disease Resistance in Plants.

Brock, N., Kaur, N., & Halford, N. G. (2025). Advances in genome editing in plants within an evolving regulatory landscape, with a focus on its application in wheat breeding. *Journal of Plant Biochemistry and Biotechnology*, 1-16.

Butler, N. M., Atkins, P. A., Voytas, D. F., & Douches, D. S. (2015). Generation and inheritance of targeted mutations in potato (Solanum tuberosum L.) using the CRISPR/Cas system. *PloS one*, *10*(12), e0144591.

Chavhan, R. L., Jaybhaye, S. G., Hinge, V. R., Deshmukh, A. S., Shaikh, U. S., Jadhav, P. K., ... & Hong, J. C. (2025). Emerging applications of gene editing technologies for the development of climate-resilient crops. *Frontiers in Genome Editing*, *7*, 1524767.

Dash, P. K., Ganesan, K. N., Manivannan, N., Kumar, S. V., Senthil, N., & Pushpam, R. (2024). Revolutionizing livestock sustainability: Pioneering breeding strategies for superior forage biomass and quality. *HORIZON*, *11*(4), 1093-1107.

Creeth, R., Thompson, A., & Kevei, Z. (2025). DNA-free CRISPR genome editing in raspberry (Rubus idaeus) protoplast through RNP-mediated transfection. *Frontiers in Genome Editing*, *7*, 1589431.

Daniel, M. A., Sebastin, R., Yu, J. K., Soosaimanickam, M. P., & Chung, J. W. (2023). Enhancing horticultural crops through genome editing: applications, benefits, and considerations. *Horticulturae*, *9*(8), 884.

Ebrahimi, V., & Hashemi, A. (2024). CRISPR-based gene editing in plants: Focus on reagents and their delivery tools. *BioImpacts: BI*, *15*, 30019.

Eckerstorfer, M. F., Grabowski, M., Lener, M., Engelhard, M., Simon, S., Dolezel, M., ... & Lüthi, C. (2021). Biosafety of genome editing applications in plant breeding: Considerations for a focused case-specific risk assessment in the EU. *BioTech*, *10*(3), 10.

Erdoğan, İ., Cevher-Keskin, B., Bilir, Ö., Hong, Y., & Tör, M. (2023). Recent developments in CRISPR/Cas9 genome-editing technology related to plant disease resistance and abiotic stress tolerance. *Biology*, *12*(7), 1037.

Faizal, A., Nugroho, S., Sembada, A. A., Theda, Y., Komariyah, T., & Esyanti, R. R. (2024). Genome editing in future crop protection: utilizing CRISPR/Cas9 to improve crop resistance against diseases, pests, and weeds. *Discover Agriculture*, *2*(1), 104.

Farinati, S., Draga, S., Betto, A., Palumbo, F., Vannozzi, A., Lucchin, M., & Barcaccia, G. (2023). Current insights and advances into plant male sterility: new precision breeding technology based on genome editing applications. *Frontiers in plant science*, *14*, 1223861.

Fiaz, S., Ahmar, S., Saeed, S., Riaz, A., Mora-Poblete, F., & Jung, K. H. (2021). Evolution and application of genome editing techniques for achieving food and nutritional security. *International journal of molecular sciences*, *22*(11), 5585.

Gallo, D., Meunier, A. C., & Périn, C. (2025). A long journey towards genome editing technologies in plants: a technical and critical review of genome editing technologies. *Frontiers in Genome Editing*, *7*, 1663352.

Gambino, G., Nuzzo, F., Moine, A., Chitarra, W., Pagliarani, C., Petrelli, A., ... & Perrone, I. (2024). Genome editing of a recalcitrant wine grape genotype by lipofectamine‐mediated delivery of CRISPR/Cas9 ribonucleoproteins to protoplasts. *The Plant Journal*, *119*(1), 404-412.

Gong, Z., Cheng, M., & Botella, J. (2021). Non-GM genome editing approaches in crops. *Frontiers in Genome Editing*. *2*(1), 104

Han, X., Deng, Z., Liu, H., & Ji, X. (2025). Current Advancement and Future Prospects in Simplified Transformation-Based Plant Genome Editing. *Plants*, *14*(6), 889.

Hao, N. T., Ben Taieb, S., Moritaka, M., & Fukuda, S. (2026). Consumer acceptance and valuation of quality‐improved food products derived by genome editing technology. A case study of rice in Vietnam. *Agribusiness*, *42*(1), 29-50.

Hoengenaert, L., Van Doorsselaere, J., Vanholme, R., & Boerjan, W. (2023). Microparticle-mediated CRISPR DNA delivery for genome editing in poplar. *Frontiers in Plant Science*, *14*, 1286663.

Huang, Z., & Liu, G. (2023). Current advancement in the application of prime editing. *Frontiers in Bioengineering and Biotechnology*, *11*, 1039315.

Hundleby, P. A., & Harwood, W. A. (2019). Impacts of the EU GMO regulatory framework for plant genome editing. *Food and energy security*, *8*(2), e00161.

Jahan, I., Angon, P. B., Mou, S. J., Zannat, M., Antu, U. B., Alam, M. M., ... & Sakil, M. A. (2025). The potential of CRISPR-Cas genome editing technologies to mitigate biotic stress in plants. *Agriculture Bioscience*, *6*(1), 0005.

Juszczuk-Kubiak, E., Mrozik, K., Piłka, N., Bąk, M., Zielińska, E., Kowalczyk, M., & Roszko, M. (2025). CRISPR genome editing technology in the agri-food industry: benefits, challenges and concerns. *Food Biotechnology and Agricultural Science*, *79*, 43-52.

Kaba, F. A., Oral, Y. B., Ata, G., Mutlu, N., & Kaba, A. (2025). Evaluation of Lettuce Genotype and Agrobacterium Strain for Transformation Efficiency Using a CRISPR-Compatible Vector. *Turkish Journal of Applied Sciences and Technology*, *6*(2), 101-110.

Kalaitzandonakes, N., Willig, C., & Zahringer, K. (2023). The economics and policy of genome editing in crop improvement. *The Plant Genome*, *16*(2), e20248.

Karanam, B. T., Kumari, V. N., Sivakumar, S., Vanitha, K., Meenakshi, P., Himakara, D. M., & Rawat, P. (2024). Nutritional enhancement and genetic innovations in sweet corn: unlocking super sweetness and health benefits through modern breeding technique–A review. *Plant Science Today*, *11*, 5441.

KEUL, A. B., Farkas, A., Carpa, R., Dobrota, C., & Iordache, D. (2022). Development of smart fruit crops by genome editing. *Turkish Journal of Agriculture and Forestry*, *46*(2), 129-140.

Kim, J. H., Yoo, C. M., Nguyen, C. D., Lee, M. B., Huo, H., & Lee, S. (2025). Optimization of Shoot Regeneration and Agrobacterium-mediated Transformation in Strawberry (Fragaria× ananassa). *Journal of the American Society for Horticultural Science*, *150*(1), 10-18.

Kumar, M., Prusty, M. R., Pandey, M. K., Singh, P. K., Bohra, A., Guo, B., & Varshney, R. K. (2023). Application of CRISPR/Cas9-mediated gene editing for abiotic stress management in crop plants. *Frontiers in Plant Science*, *14*, 1157678.

Kumar, U., Dwivedi, D., & Das, U. (2025). Advancements in CRISPR‐Mediated Multiplex Genome Editing: Transforming Plant Breeding for Crop Improvement and Polygenic Trait Engineering. *Biotechnology Journal*, *20*(11), e70148.

Lassoued, R., Macall, D. M., Hesseln, H., Phillips, P. W., & Smyth, S. J. (2019). Benefits of genome-edited crops: expert opinion. *Transgenic research*, *28*(2), 247-256.

Li, J., Zhang, C., He, Y., Li, S., Yan, L., Li, Y., ... & Xia, L. (2023). Plant base editing and prime editing: the current status and future perspectives. *Journal of Integrative Plant Biology*, *65*(2), 444-467.

Li, Y., Liang, J., Deng, B., Jiang, Y., Zhu, J., Chen, L., ... & Li, J. (2023). Applications and prospects of CRISPR/Cas9-mediated base editing in plant breeding. *Current Issues in Molecular Biology*, *45*(2), 918-935.

Ma, F., Zheng, Q., Sun, Y., Zhang, N., & Xu, W. (2025). Toward Tissue-Free Plant Engineering: Emerging Platforms for Sustainable Horticultural Transformation. *Journal of Agricultural and Food Chemistry*, *73*(42), 26457-26471.

Malnoy, M., Viola, R., Jung, M. H., Koo, O. J., Kim, S., Kim, J. S., ... & Nagamangala Kanchiswamy, C. (2016). DNA-free genetically edited grapevine and apple protoplast using CRISPR/Cas9 ribonucleoproteins. *Frontiers in plant science*, *7*, 1904.

Mamrutha, H. M., Zeenat, W., Kapil, D., Budhagatapalli, N., Tikaniya, D., Rakesh, K., ... & Singh, G. P. (2024). Evidence and opportunities for developing non-transgenic genome edited crops using site-directed nuclease 1 approach. *Critical Reviews in Biotechnology*, *44*(6), 1140-1150.

Mann, A., Ranga, P., Choudhary, P., Yadav, S., Kaul, N., Dahiya, A., ... & Sanwal, S. K. (2024). Genome editing technologies for enhancing plant resilience to biotic and abiotic stresses-brief review. *Journal of Soil Salinity and Water Quality*, *16*(2), 180-193.

Manzoor, S., Nabi, S. U., Rather, T. R., Gani, G., Mir, Z. A., Wani, A. W., ... & Manzar, N. (2024). Advancing crop disease resistance through genome editing: a promising approach for enhancing agricultural production. *Frontiers in genome editing*, *6*, 1399051.

McLaughlin, J. E., Kue Foka, I. C., Lawton, M. A., & Di, R. (2025). CRISPR activation: identifying and using novel genes for plant disease resistance breeding. *Frontiers in Genome Editing*, *7*, 1596600.

Mitchell, J. (2025). CRISPR-Cas9-Mediated Crop Genome Editing: From Basic Research to Breeding Applications. *Plant Scientific Application*, *1*(1), 15-21.

Modrzejewski, D., Hartung, F., Sprink, T., Krause, D., Kohl, C., & Wilhelm, R. (2019). What is the available evidence for the range of applications of genome-editing as a new tool for plant trait modification and the potential occurrence of associated off-target effects: a systematic map. *Environmental Evidence*, *8*(1), 1-33.

Mushtaq, M., Sakina, A., Wani, S. H., Shikari, A. B., Tripathi, P., Zaid, A., ... & Salgotra, R. K. (2019). Harnessing genome editing techniques to engineer disease resistance in plants. *Frontiers in plant science*, *10*, 550.

Nakazato, I., Yamori, W., Matsumura, H., Qu, Y., Okuno, M., Tsutsumi, N., & Arimura, S. I. (2025). Resistance to the herbicide metribuzin conferred to Arabidopsis thaliana by targeted base editing of the chloroplast genome. *Plant Biotechnology Journal*, *23*(1), 204-215.

Namata, M. J., Xu, J., Habyarimana, E., Palakolanu, S. R., Wang, L., & Li, J. (2025). Genome editing in maize and sorghum: A comprehensive review of CRISPR/Cas9 and emerging technologies. *The Plant Genome*, *18*(2), e70038.

Niraula, S., Khanal, R., & Ghimire, N. (2024). CRISPR-Cas based precision genome editing: current advances and associated challenges in crop improvement and trait enhancement. *Genetics & Applications*, *8*(2).

Nonaka, S., & Ezura, H. (2024). Possibility of genome editing for melon breeding. *Breeding Science*, *74*(1), 47-58.

Odipio, J., Alicai, T., Ingelbrecht, I., Nusinow, D. A., Bart, R., & Taylor, N. J. (2017). Efficient CRISPR/Cas9 genome editing of phytoene desaturase in cassava. *Frontiers in plant science*, *8*, 1780.

Pak, S., & Li, C. (2022). Progress and challenges in applying CRISPR/Cas techniques to the genome editing of trees. *Forestry Research*, *2*, 6.

Priyanka, K., Shourabh, J., Maya, B., Bahadur, S. D., Jayadurga, N. R., & Ayesha, S. (2025). CRISPR-Cas9 Applications in Horticultural Crop Improvement. *Plant Cell Biotechnology and Molecular Biology*.

Qin, Y., Hua, S., Zhu, L., Nie, P., Yang, C., Xu, F., ... & Dong, W. (2025). Application of graphene oxide in Agrobacterium-mediated genetic transformation and construction of a novel DNA delivery system for watermelon. *Scientific Reports*, *15*(1), 5465.

Rivera-Toro, D. M., de Folter, S., & Alvarez-Venegas, R. (2025). CRISPR/dCas12a-mediated activation of SlPAL2 enhances tomato resistance against bacterial canker disease. *PloS one*, *20*(3), e0320436.

Ruffell, D. (2018). The EU Court of Justice extends the GMO Directive to gene‐edited organisms. *FEBS letters*, *592*(22), 3653-3657.

Sahab, S., Runa, F., Ponnampalam, M., Kay, P. T., Jaya, E., Viduka, K., ... & Hayden, M. J. (2024). Efficient multi-allelic genome editing via CRISPR–Cas9 ribonucleoprotein-based delivery to Brassica napus mesophyll protoplasts. *Frontiers in Plant Science*, *15*, 1397632.

Sahu, S. P., Joshi, R. K., & Mishra, R. (2024). Genome editing technologies towards tomato improvement: Recent advances and future perspectives. *Journal of Experimental Biology and Agricultural Sciences*, *12*(4), 537-556.

Salvagnin, U., Unkel, K., Sprink, T., Bundock, P., Sevenier, R., Bogdanović, M., ... & Malnoy, M. (2023). A comparison of three different delivery methods for achieving CRISPR/Cas9 mediated genome editing in Cichorium intybus L. *Frontiers in plant science*, *14*, 1111110.

Sampath, V., Rangarajan, N., CH, S., Deori, M., Veeraragavan, M., Ghodake, B. D., & Kaushal, K. (2023). Advancing crop improvement through CRISPR technology in precision agriculture trends-a review. *International Journal of Environment and Climate Change*, *13*(11), 4683-4694.

Sharma, K. K., Palakolanu, S. R., Bhattacharya, J., Shankhapal, A. R., & Bhatnagar-Mathur, P. (2022). CRISPR for accelerating genetic gains in under-utilized crops of the drylands: Progress and prospects. *Frontiers in Genetics*, *13*, 999207.

Sheri, V., Mohan, H., Jogam, P., Alok, A., Rohela, G. K., & Zhang, B. (2025). CRISPR/Cas genome editing for cotton precision breeding: mechanisms, advances, and prospects. *Journal of Cotton Research*, *8*(1), 4.

Shivashakarappa, K., Marriboina, S., Dumenyo, K., Taheri, A., & Yadegari, Z. (2025). Nanoparticle-mediated gene delivery techniques in plant systems. *Frontiers in Nanotechnology*, *7*, 1516180.

Shrawat, A. K., & Armstrong, C. L. (2018). Development and application of genetic engineering for wheat improvement. *Critical Reviews in Plant Sciences*, *37*(5), 335-421.

Smyth, S. J. (2019). Global status of the regulation of genome editing technologies. *CABI Reviews*, (2019), 1-6.

Son, S., & Park, S. R. (2022). Challenges facing CRISPR/Cas9-based genome editing in plants. *Frontiers in plant science*, *13*, 902413.

Sprink, T., Eriksson, D., Schiemann, J., & Hartung, F. (2016). Regulatory hurdles for genome editing: process-vs. product-based approaches in different regulatory contexts. *Plant cell reports*, *35*(7), 1493-1506.

Sprink, T., Wilhelm, R., & Hartung, F. (2022). Genome editing around the globe: an update on policies and perceptions. *Plant Physiology*, *190*(3), 1579-1587.

Srivastava, S. (2025). Molecular Regulation of Shoot and Root Apical Meristems-Advances and Applications in Crop Improvement. *NewBioWorld*, *7*(1), 31-38.

Mehandi, S., Harshavardhan, S. S., Metta, B., & Abhimanyu, G. (2025). Genomic Tools in Mustard Breeding for the Enhancement of Yield, Oil Quality and Stress Tolerance. *International Journal of Plant & Soil Science*, *37*(3), 362-376.

Tadkal, R., Susmitha, P., Tejaswini, R., Neresha, W., Venkatesh, C., Tejasree, P., ... & Behera, H. S. (2024). Genetic engineering and vegetable crop improvement: a review. *Plant Archives (09725210)*, *24*(2).

Tamizi, A. A., Zainal-Abidin, R. A., Md-Yusof, A. A., Mohd-Zim, N. A., Salleh, M. S., Azmi, N. S. A., ... & Samsulrizal, N. H. (2025). Genome Editing for Enhanced Abiotic Stress Tolerance in Selected Cereal (Poaceae) Crops: Current Applications, Tools, and Future Perspectives. *Malaysian Applied Biology*, *54*(4), 1-24.

Tian, S., Yao, L., Zhang, Y., Rao, X., & Zhu, H. (2025). Prime editing for crop improvement: a systematic review of optimization strategies and advanced applications. *Genes*, *16*(8), 965.

Toda, E., Kato, N., Higashiyama, T., & Okamoto, T. (2023). Genome editing approaches using reproductive cells/tissues in flowering plants. *Frontiers in Genome Editing*, *4*, 1085023.

Tripathi, J. N., & Tripathi, L. (2024). Using CRISPR-Cas9 genome editing to enhance disease resistance in banana. *CABI Reviews*, *19*(1).

Tripathi, L., Dhugga, K. S., Ntui, V. O., Runo, S., Syombua, E. D., Muiruri, S., ... & Tripathi, J. N. (2022). Genome editing for sustainable agriculture in Africa. *Frontiers in Genome Editing*, *4*, 876697.

Tripathi, L., Ntui, V. O., Tripathi, J. N., Norman, D., & Crawford, J. (2023). A new and novel high‐fidelity genome editing tool for banana using Cas‐CLOVER. *Plant Biotechnology Journal*, *21*(9), 1731.

Ukhatova, Y. V., Erastenkova, M. V., Korshikova, E. S., Krylova, E. A., Mikhailova, A. S., Semilet, T. V., ... & Khlestkina, E. K. (2023). Improvement of crops using the CRISPR/Cas system: new target genes. *Molecular Biology*, *57*(3), 375-397.

Uranga, M., & Daròs, J. A. (2023). Tools and targets: The dual role of plant viruses in CRISPR–Cas genome editing. *The Plant Genome*, *16*(2), e20220.

Valencia-Lozano, E., Cabrera-Ponce, J. L., Barraza, A., López-Calleja, A. C., García-Vázquez, E., Rivera-Toro, D. M., ... & Alvarez-Venegas, R. (2024). Editing of SlWRKY29 by CRISPR-activation promotes somatic embryogenesis in Solanum lycopersicum cv. Micro-Tom. *Plos one*, *19*(4), e0301169.

Veillet, F., Perrot, L., Chauvin, L., Kermarrec, M. P., Guyon-Debast, A., Chauvin, J. E., ... & Mazier, M. (2019). Transgene-free genome editing in tomato and potato plants using agrobacterium-mediated delivery of a CRISPR/Cas9 cytidine base editor. *International Journal of Molecular Sciences*, *20*(2), 402.

Vu, T. V., Nguyen, N. T., Kim, J., Das, S., Lee, J., & Kim, J. Y. (2022). The obstacles and potential solution clues of prime editing applications in tomato. *BioDesign Research*, *2022*, 0001.

Vu, T. V., Nguyen, N. T., Kim, J., Hong, J. C., & Kim, J. Y. (2024). Prime editing: mechanism insight and recent applications in plants. *Plant Biotechnology Journal*, *22*(1), 19-36.

Waghmode, P., Pitha, C. C., & Banka, V. (2024). The role of genome editing technologies in sustainable agriculture.

Journal of Advances in Biology Biotechnology Biotechnological Equipment, *27*(8), 543-558.

Wang, Y., Zafar, N., Ali, Q., Manghwar, H., Wang, G., Yu, L., ... & Jin, S. (2022). CRISPR/Cas genome editing technologies for plant improvement against biotic and abiotic stresses: advances, limitations, and future perspectives. *Cells*, *11*(23), 3928.

Whelan, A. I., Gutti, P., & Lema, M. A. (2020). Gene editing regulation and innovation economics. *Frontiers in Bioengineering and Biotechnology*, *8*, 303.

Wolt, J. D., & Wolf, C. (2018). Policy and governance perspectives for regulation of genome edited crops in the United States. *Frontiers in Plant Science*, *9*, 1606.

Aqib, Z. E. B., Ahmad, S., Tabbasum, J., Sheng, Z., & Peisong, H. U. (2022). Rice grain yield and quality improvement via CRISPR/Cas9 system: an updated review. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca*, *50*(3), 12388-12388.

Zegeye, W. A., Tsegaw, M., Zhang, Y., & Cao, L. (2022). CRISPR-based genome editing: advancements and opportunities for rice improvement. *International Journal of Molecular Sciences*, *23*(8), 4454.

Zhang, Y., Cheng, Y., Fang, H., Roberts, N., Zhang, L., Vakulskas, C. A., ... & Qi, Y. (2022). Highly efficient genome editing in plant protoplasts by ribonucleoprotein delivery of CRISPR-Cas12a nucleases. *Frontiers in Genome Editing*, *4*, 780238.

Zhang, Y., Restall, J., Crisp, P., Godwin, I., & Liu, G. (2021). Current status and prospects of plant genome editing in Australia. *In Vitro Cellular & Developmental Biology-Plant*, *57*(4), 574-583.