SUNSHINE AGRICULTURE
e-Newsletter

ISSN (E): 2583 – 0821

Sun. Agri.:e- Newsletter, (2026) *6*(7), 33-37

Article ID: 620

CRISPR-Based Genome Editing in Crop Improvement

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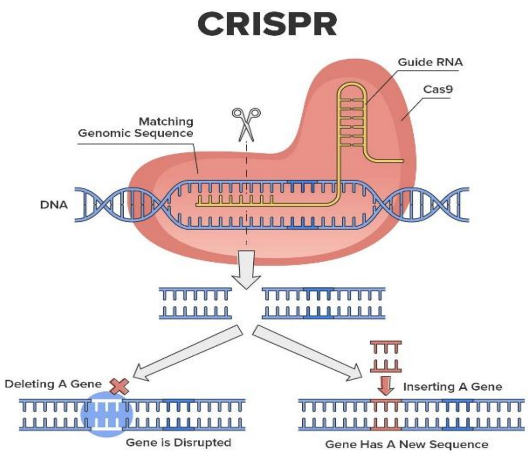
Available online at
www.sunshineagriculture.vitalbiotech.org

Article History
Received: 05.07.2026
Revised: 10.07.2026
Accepted: 15.07.2026

INTRODUCTION

Agriculture is under unprecedented pressure to meet the food demands of a rapidly growing global population while simultaneously coping with climate change, declining arable land, emerging pests and diseases, and environmental degradation. Traditional plant breeding has contributed substantially to crop improvement over the past century; however, the process often requires several years or decades to develop improved varieties. Moreover, conventional breeding depends on naturally occurring genetic variation and is constrained by sexual compatibility barriers among species. Genetic engineering has helped overcome some of these limitations by introducing desirable genes into crop plants. Nevertheless, concerns regarding transgenic crops, biosafety regulations, and public acceptance have limited widespread adoption in certain regions.

Genome editing has emerged as a transformative technology that enables precise modification of specific DNA sequences within the genome. Among various genome editing tools, CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) and CRISPR-associated (Cas) proteins have gained enormous attention because of their simplicity, efficiency, versatility, and cost-effectiveness. Since its adaptation from a bacterial immune defense system into a genome editing tool in 2012, CRISPR technology has rapidly become one of the most powerful tools in plant biotechnology. Unlike earlier genome editing technologies such as Zinc Finger Nucleases (ZFNs) and Transcription Activator-Like Effector Nucleases (TALENs), CRISPR relies on RNA-guided DNA recognition, making it easier to design and implement.



The application of CRISPR technology in crop improvement has enabled the development of varieties with enhanced yield, improved nutritional quality, resistance to biotic and abiotic stresses, and superior agronomic traits. Furthermore, recent advances such as base editing, prime editing, multiplex editing, and epigenome editing have expanded the possibilities for precise and predictable genome manipulation. Consequently, CRISPR-based genome editing is becoming a cornerstone of modern plant breeding and sustainable agriculture.

2. History and Evolution of Genome Editing Technologies

The development of genome editing technologies has evolved through several generations:

Table 1: Evolution of Genome Editing Technologies

Technology	Period	Major Characteristics
Mutagenesis	1950s–1980s	Random mutations
Genetic Engineering	1980s–2000s	Transgenic crops
ZFNs	1990s	Protein-based targeting
TALENs	2010	Improved specificity
CRISPR-Cas9	2012	RNA-guided editing
Base Editing	2016	Single nucleotide editing
Prime Editing	2019	Precise DNA rewriting

CRISPR technology represents a major advancement due to its simplicity, efficiency, and ability to target multiple genes simultaneously.

3. CRISPR System: Origin and Biological Basis

CRISPR was first discovered in bacteria and archaea as an adaptive immune system against invading viruses and plasmids. The system consists of repeated DNA sequences separated by spacer sequences derived from foreign genetic material.

Components of CRISPR System

- CRISPR array
- Cas proteins
- Guide RNA (gRNA)
- Protospacer Adjacent Motif (PAM)

The CRISPR-Cas system recognizes foreign DNA and cleaves it through RNA-guided mechanisms.

4. Mechanism of CRISPR-Cas9 Genome Editing

CRISPR-Cas9 genome editing is a revolutionary molecular technology that enables precise modification of specific DNA sequences within an organism's genome. The system is derived from the natural adaptive immune defense mechanism found in bacteria and archaea, where it protects against invading viruses and foreign genetic elements. In crop improvement, the CRISPR-Cas9 system primarily consists of two key components: a guide RNA (gRNA) and the Cas9 nuclease enzyme. The guide RNA is designed to be complementary to a specific target DNA sequence, thereby directing the Cas9 protein to the desired location in the genome. Once the guide RNA binds to the target DNA adjacent to a Protospacer Adjacent Motif (PAM) sequence, the Cas9 enzyme introduces a precise double-stranded break (DSB) at the target site. Following DNA cleavage, the plant cell activates its natural DNA repair mechanisms to repair the break. Two major repair pathways are involved: Non-Homologous End Joining (NHEJ) and Homology-Directed Repair (HDR). NHEJ is an error-prone process that often introduces small insertions or deletions (indels), resulting in gene disruption or knockout. In contrast, HDR utilizes a donor DNA template to introduce precise genetic modifications, such as gene replacement or targeted insertion of desirable traits. Through these mechanisms, CRISPR-Cas9 can be used to deactivate undesirable genes, enhance beneficial traits, correct mutations, and create novel genetic variations. The simplicity, accuracy, efficiency, and versatility of CRISPR-Cas9 have made it one of the most powerful tools for modern crop breeding, enabling the rapid development of improved crop varieties with enhanced yield, stress tolerance, nutritional quality, and resistance to pests and diseases.

5. Types of CRISPR Systems

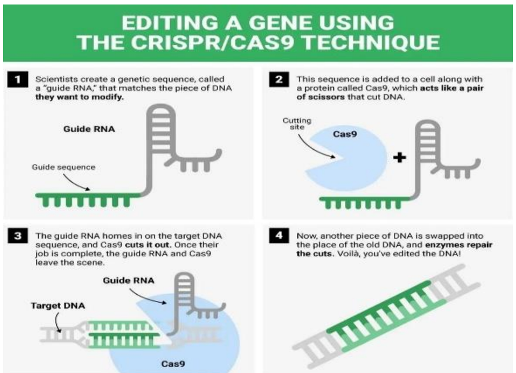
The CRISPR-Cas system has evolved into a diverse family of genome editing tools, each possessing unique characteristics and applications in crop improvement. Based on their structural and functional differences, CRISPR systems are broadly classified into two classes and multiple types. Among these, CRISPR-Cas9 remains the most widely used system due to its simplicity and high editing efficiency. Cas9 functions as an RNA-guided DNA endonuclease capable of generating double-strand breaks at specific genomic locations, making it highly suitable for gene knockout and targeted mutagenesis. Another important system is CRISPR-Cas12a (Cpf1), which differs from Cas9 in recognizing a different PAM sequence and producing staggered DNA cuts. Cas12a offers advantages such as multiplex genome editing, simpler guide RNA requirements, and improved specificity. CRISPR-Cas13 represents a unique RNA-targeting system that cleaves RNA molecules rather than DNA, making it particularly useful for combating RNA viruses and regulating gene expression. Additionally, catalytically inactive Cas proteins, known as dead Cas9 (dCas9), are employed for transcriptional regulation, epigenome editing, and gene activation or repression without altering DNA sequences. More recently, Cas14 and other miniature CRISPR systems have been developed for highly specific nucleic acid detection and diagnostic applications. Continuous discoveries of novel Cas proteins and engineered variants are expanding the capabilities of genome editing, enabling researchers to address increasingly complex breeding objectives. These diverse CRISPR platforms provide unprecedented opportunities for precision breeding and functional genomics in agricultural crops.

6. Base Editing Technologies

Base editing represents a major advancement in genome engineering that allows precise conversion of one DNA base into another without creating double-stranded DNA breaks or requiring donor DNA templates. Traditional CRISPR-Cas9 editing relies on cellular repair mechanisms following DNA cleavage, which can sometimes result in unintended mutations. Base editing overcomes this limitation by

ISSN (E): 2583 – 0821

directly altering individual nucleotides with high precision. The technology combines a catalytically impaired Cas protein with a nucleotide deaminase enzyme, enabling targeted chemical modification of specific DNA bases. Two major classes of base editors have been developed: Cytosine Base Editors (CBEs), which convert cytosine (C) to thymine (T), and Adenine Base Editors (ABEs), which convert adenine (A) to guanine (G). These targeted nucleotide substitutions enable correction of point mutations and modification of genes controlling important agronomic traits. In crop improvement, base editing has been successfully used to develop herbicide-resistant rice and wheat varieties, improve disease resistance, modify flowering time, enhance grain quality, and increase stress tolerance. Because a large proportion of naturally occurring genetic variations involve single nucleotide polymorphisms (SNPs), base editing provides an efficient approach for recreating beneficial alleles without introducing foreign DNA. Furthermore, base editing minimizes unintended genomic alterations, thereby improving editing accuracy and reducing regulatory concerns. As a result, base editing is increasingly recognized as a valuable tool for precision breeding and next-generation crop improvement.



7. Prime Editing

Prime editing is a recently developed genome editing technology that significantly expands the precision and versatility of CRISPR-based genetic modifications. Often described as a "search-and-replace" genome editing system, prime editing enables targeted insertions, deletions, and all possible base substitutions without requiring double-strand DNA breaks or donor DNA templates. The system utilizes a fusion protein consisting of a modified Cas9 nickase and a reverse transcriptase enzyme, guided by a specialized prime editing guide RNA (pegRNA). The pegRNA not only directs the editing machinery to the target site but also contains the desired genetic information to be incorporated into the genome. Once the target DNA is recognized, the Cas9 nickase introduces a single-strand break, and the reverse transcriptase synthesizes the desired sequence directly into the genome using the pegRNA template. This process allows highly precise editing with reduced risks of unwanted insertions, deletions, and off-target mutations.

ISSN (E): 2583 – 0821

Prime editing has enormous potential for crop improvement because it can correct deleterious mutations, introduce beneficial alleles, and engineer complex genetic traits that are difficult to achieve using conventional genome editing methods. Researchers have successfully applied prime editing in rice, wheat, maize, tomato, and several other crops to modify genes associated with yield, disease resistance, stress tolerance, and nutritional quality. Compared with earlier genome editing technologies, prime editing offers greater flexibility and precision while minimizing genomic damage. Although challenges related to editing efficiency and delivery methods remain, ongoing improvements in prime editing systems are expected to accelerate their adoption in plant breeding programs. As the technology matures, prime editing is likely to play a central role in developing next-generation crop varieties capable of meeting future agricultural and food security challenges.

8. CRISPR for Disease Resistance

Table 4: Disease Resistance Through CRISPR

Crop	Disease	Edited Gene
Rice	Bacterial blight	SWEET genes
Wheat	Powdery mildew	MLO
Tomato	TYLCV	eIF4E

CONCLUSION

CRISPR-based genome editing has revolutionized crop improvement by providing a precise, efficient, and versatile platform for modifying plant genomes. The technology offers unprecedented opportunities for developing high-yielding, climate-resilient, pest- and disease-resistant, nutritionally enhanced crop varieties capable of addressing future food security challenges. Advances such as base editing, prime editing, multiplex editing, and epigenome engineering have further expanded the scope of genome manipulation beyond traditional breeding and transgenic approaches. Successful applications across cereals, pulses, oilseeds, fruits, vegetables, and ornamental crops demonstrate the transformative potential of CRISPR technology in agriculture. Nevertheless, challenges related to off-target effects, regulatory frameworks, biosafety assessments, and public acceptance require careful consideration. Continued integration of CRISPR with genomics, artificial intelligence, synthetic biology, and precision agriculture will accelerate the development of next-generation crops adapted to climate change and sustainable farming systems. As research advances and regulatory clarity improves, CRISPR-based genome editing is expected to become a cornerstone of future crop breeding programs and global agricultural innovation.