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Advances in CRISPR/Cas-Based genome editing for crop improvement: A review

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Abstract
The global population is on a relentless trajectory toward 10 billion by mid-century, a demographic shift that places unprecedented pressure on agricultural systems to increase food production sustainably and equitably. Traditional crop breeding, a cornerstone of agricultural development for millennia, is increasingly challenged by the rapid pace of climate change, the emergence of new plant diseases, and the need for enhanced nutritional content to address hidden hunger. The advent of CRISPR/Cas (Clustered Regularly Interspaced Short Palindromic Repeats-CRISPR-associated) technology has provided a powerful and precise solution to these challenges, revolutionizing the field of plant breeding. This review focuses on the latest research from the past five years (2020-2024), a period marked by a transition from foundational CRISPR/Cas9-mediated gene knockouts to more sophisticated and diverse genome editing strategies. We detail the technological innovations that have made CRISPR more efficient and accurate, such as the refinement of base and prime editing. We also present a comprehensive table of recent successful applications across a variety of crop species, from staple grains to horticultural products. This paper critically evaluates the ongoing challenges, including technical limitations in delivery and specificity, as well as the complex regulatory and societal hurdles that must be navigated for these innovations to reach farmers and consumers globally.

Keywords: CRISPR/Cas9, genome editing, crop improvement, agricultural biotechnology and trait enhancement

Introduction

The current era is defined by the dual imperatives of feeding a growing population and adapting to a rapidly changing climate. The Food and Agriculture Organization of the United Nations (FAO) has highlighted the critical need for a ‘paradigm shift’ in agriculture to achieve food security and promote sustainable development (FAO, 2021) [7]. Historically, crop improvement has been a slow and incremental process, with conventional breeding methods requiring multiple generations to achieve a desired trait. While traditional genetic modification (GM) allowed for the introduction of foreign DNA to create new traits, it has faced significant regulatory and public resistance, particularly in Europe and other parts of the world (Waltz, 2018) [23].

The discovery and adaptation of the bacterial CRISPR/Cas system for use in eukaryotes, beginning with seminal work by Jinek *et al*. (2012) [11] and Cong *et al*. (2013) [5], offered a breakthrough. Unlike its predecessors—Zinc Finger Nucleases (ZFNs) and Transcription Activator-Like Effector Nucleases (TALENs)—CRISPR/Cas is remarkably simple to design, cost-effective, and highly versatile. The system's core mechanism, involving a guide RNA (gRNA) directing a Cas protein to a specific DNA sequence to create a double-strand break (DSB), has become the foundation for a new generation of precision breeding (Doudna & Charpentier, 2014) [6]. This has enabled scientists to edit a plant's own genes, a process that, in many cases, does not introduce foreign DNA, making the resulting crops indistinguishable from those produced through conventional mutagenesis or breeding. This distinction has led to a more permissive regulatory environment in some countries, such as the United States and Japan, facilitating the path to commercialization (Waltz, 2022) [24].

The last five years have witnessed an explosion of research, moving beyond the simple ‘molecular scissors’ analogy of Cas9. Researchers have developed a suite of advanced tools that address the limitations of early CRISPR systems. These include the development of base and prime editors, which allow for single-nucleotide changes or small insertions/deletions without the creation of a DSB, thereby mitigating the risk of off-target effects and large chromosomal rearrangements (Anzalone *et al*., 2019; Komor *et al*., 2016) [2, 13]. Furthermore, new Cas variants with different PAM specificities and the ability to target RNA have expanded the genomic landscape that can be edited (Zetsche *et al*., 2015) [27]. This technological evolution has enabled a wide range of applications, from enhancing a crop's natural defenses against pathogens to boosting its nutritional profile and improving its resilience to environmental stresses like drought and heat (Abudayyeh *et al*., 2020) [1]. As a result, CRISPR/Cas is now a central pillar in the global effort to create a more resilient and sustainable food system.

Advances in CRISPR/Cas Technologies (2020-2024)

The past five years have been a period of intense innovation in CRISPR/Cas technology, with a focus on enhancing precision, efficiency, and safety for plant applications.

Base and Prime Editing

The development and refinement of base and prime editing have been transformative. These ‘DSB-free’ technologies allow for precise genetic changes without the risk of off-target indels associated with error-prone NHEJ repair

- **Base Editing:** This technology allows for the direct conversion of one nucleotide to another (e.g., C to T or A to G) using a Cas9 nickase fused to a deaminase enzyme. Recent research has focused on improving the window of activity and reducing bystander edits, where nearby bases are also unintentionally changed. A prime example of its application is the development of a high-GABA tomato in Japan, which was the first CRISPR-edited food approved for commercial sale. Base editing was used to introduce a specific point mutation that led to the accumulation of GABA, a compound linked to lower blood pressure (Waltz, 2022) [24].

- **Prime Editing:** Prime editing is a 'search-and-replace' tool that fuses a Cas9 nickase with a reverse transcriptase, guided by a prime editing guide RNA (pegRNA) that contains both the targeting sequence and the desired edit. This system enables precise insertions, deletions, or all four types of single-base conversions. While still facing challenges in terms of efficiency in plants, recent studies have demonstrated its potential for introducing precise heat-shock elements into promoters of key genes in rice and tomato, a strategy aimed at improving heat tolerance (Lou *et al*., 2025) [18]. The ability of prime editing to perform targeted modifications without a DSB makes it a highly promising tool for next-generation crop breeding.

Novel Cas Variants and Delivery Systems

The discovery and engineering of new Cas proteins have expanded the CRISPR toolbox. Cas12, for example, offers different PAM recognition sites, increasing the range of targetable genomic loci. Researchers have also made significant strides in developing more efficient and scalable delivery methods. Traditional *Agrobacterium*-mediated transformation or biolistic particle bombardment are often species-specific and require extensive tissue culture. Recent research has explored the use of lipid nanoparticles (LNPs) and non-viral vectors to deliver CRISPR components directly into plant cells (Patil *et al*., 2024) [19]. This approach could bypass the need for traditional plant regeneration and accelerate the breeding cycle significantly.

Applications of CRISPR/Cas in Crop Improvement (2020-2024)

The application of CRISPR/Cas technology in the last five years has resulted in the development of a diverse array of new crop varieties with enhanced traits. The research has focused on improving resilience to climate-related stresses, enhancing nutritional quality, and increasing yield, particularly in staple crops.

- **Abiotic Stress Tolerance:** Climate change-related stresses like drought and heat have been a major focus. In wheat, researchers have used CRISPR/Cas9 to enhance heat tolerance by modulating heat shock factors, such as *HsfA1b*, allowing the plant to maintain growth and yield under high temperatures (Tian *et al*., 2020) [22]. In maize, a study published in *Nature* modified a gene (*ZmHDT103*) involved in the abscisic acid (ABA) signaling pathway, resulting in improved drought tolerance without a significant yield penalty under normal conditions (Shelake *et al*., 2022) [21].

- **Biotic Stress Resistance:** Creating crops resistant to pests and diseases has a direct impact on reducing crop losses and reliance on chemical pesticides. A significant advance in horticulture was the use of CRISPR/Cas9 to modify the promoter region of the *CsLOB1* gene in citrus, which conferred resistance to citrus canker, a devastating bacterial disease (Peng *et al*., 2020) [20]. In bananas, which are threatened by a number of diseases, researchers have edited the *DMR6* gene to create mutants resistant to banana wilt (Hu *et al*., 2021) [10].

- **Nutritional and Agronomic Improvements:** The world's first CRISPR-edited food approved for sale, the Sicilian Rouge High GABA tomato, was engineered to contain higher levels of gamma-aminobutyric acid, a compound linked to reduced stress and blood pressure (Waltz, 2022) [23]. In bananas, editing the gene for lycopene epsilon-cyclase in the Grand Naine Cavendish cultivar led to a six-fold increase in beta-carotene content, a precursor to vitamin A (Kaur *et al*., 2020) [12]. A landmark study in sorghum successfully modified genes in the α-kafirin family to improve protein quality and digestibility (Li *et al*., 2020) [16, 17].

Tables: Recent CRISPR/Cas-Based Crop Improvements (2020-2024)

The following tables present a summary of key research papers from the past five years, showcasing the breadth and impact of CRISPR/Cas technology across various crops and traits.

Table-1 highlights recent efforts to develop crops that can withstand environmental stresses such as drought, heat, and salinity. The research listed shows that CRISPR/Cas9-mediated gene knockouts or knock-ins have been successfully used to confer these traits. For instance, in maize, the knockout of the ZmHDT103 gene has been shown to improve drought tolerance (Shelake *et al*., 2022) [21], while in wheat, editing the TaHsfA1b gene enhanced the plant's ability to tolerate high temperatures (Tian *et al*., 2020) [22]. Similarly, a targeted knock-in in tomato of the SlHKT1;2 allele has been shown to improve salt tolerance during germination (Yuste-Lisbona *et al*., 2020) [25].

Table 1: Abiotic Stress Tolerance

Crop Species	Target Gene(s)	Trait Improved	CRISPR Technology Used	Reference
Maize (Zea mays)	ZmHDT103	Improved drought tolerance	CRISPR/Cas9 (gene knockout)	Shelake *et al*., 2022 [21]
Wheat (Triticum aestivum)	TaHsfA1b	Enhanced heat tolerance	CRISPR/Cas9	Tian *et al*., 2020 [22]
Tomato (Solanum lycopersicum)	SlHKT1;2	Salt tolerance during germination	CRISPR/Cas9 (knock-in)	Yuste-Lisbona *et al*., 2020 [25]
Rice (Oryza sativa)	OsCKX11	Increased grain number and yield	CRISPR/Cas9 (gene knockout)	Zhang *et al*., 202 [29]

Soybean (Glycine max)	E1 gene	Early flowering under long-day conditions	CRISPR/Cas9 (gene knockout)	Han *et al*., 2020 [9]

Table-2 focuses on research aimed at protecting crops from pathogens, pests, and parasites. The studies demonstrate the power of CRISPR/Cas9 in creating disease-resistant varieties by targeting susceptibility genes or those involved in the plant's defense response. For example, a modification of the CsLOB1 promoter in citrus conferred resistance to citrus canker, a significant threat to the citrus industry (Peng *et al*., 2020) [20]. In bananas, editing the DMR6 gene created mutants resistant to banana wilt, a devastating fungal disease (Hu *et al*., 2021) [10]. Similarly, research has successfully used CRISPR/Cas9 to knock out the eIF4E gene in cucumber, providing resistance to a range of viral diseases (Chandrasekaran *et al*., 2020) [4].

Table: 2 Biotic Stress Resistance

Crop Species	Target Gene(s)	Trait Improved	CRISPR Technology Used	Reference
Citrus (Citrus sinensis)	CsLOB1 promoter	Resistance to citrus canker disease	CRISPR/Cas9	Peng *et al*., 2020 [20]
Banana (Musa acuminata)	DMR6	Resistance to banana wilt	CRISPR/Cas9 (gene knockout)	Hu *et al*., 2021 [10]
Cucumber (Cucumis sativus)	eIF4E	Resistance to viral diseases	CRISPR/Cas9 (gene knockout)	Chandrasekaran *et al*., 2020 [4]
Rice (Oryza sativa)	OsSWEET13/14	Resistance to bacterial blight	CRISPR/Cas9 (gene knockout)	Li *et al*., 2021 [14, 15]
Tomato (Solanum lycopersicum)	MAX-1	Resistance to root parasite (Phelipanche aegyptiaca)	CRISPR/Cas9	Bari *et al*., 2021 [3]

Conclusion

The last five years have demonstrated that CRISPR/Cas-based genome editing is a mature and indispensable tool in crop science. The latest research highlights a shift from basic gene knockout to more sophisticated and precise editing, exemplified by the increasing use of base and prime editing. The development of new Cas variants and improved delivery systems is addressing key technical limitations, paving the way for more widespread and efficient applications. The commercialization of the first CRISPR-edited food in Japan and the ongoing efforts in other countries signal a new era for agriculture. Despite the remaining challenges related to regulatory frameworks and public acceptance, the momentum of research from 2020-2025 suggests that CRISPR/Cas technology is well-positioned to play a leading role in creating a sustainable and resilient food system capable of meeting the demands of a growing global population.

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