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Review Article

Unlocking the green revolution CRISPR-cas9 and the future of sustainable plant breeding: A review

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Abstract
Agriculture underwent significant changes with the Green Revolution, which substantially increased food production; however, with the global population projected to reach 11.2 billion by 2050, the demand for food is expected to continue rising. Researchers are employing novel technologies, such as Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) and CRISPR-associated protein 9 (CRISPR-Cas9) genome editing, to address this issue. The information is gathered using keywords such as "green revolution," "CRISPR-Cas9," "sustainable agriculture," "gene editing," and "crop improvement" from authenticated sources, including Google Scholar, PubMed, and NCBI. This review explores the potential of CRISPR-Cas9 to unlock new possibilities for crop improvement, with a focus on enhancing resistance to pests and diseases. Creating crops with inbuilt defenses could reduce chemical pesticide use, improving environmental and human health. Increased stress tolerance can be achieved by genetically altering crops to resist heat, salt, drought, and other environmental challenges, thereby ensuring food security. CRISPR-Cas9 also enables biofortification, enhancing crops with essential vitamins and minerals, thereby addressing dietary inadequacies and malnutrition. Introducing genes that enable nitrogen fixation within crops may reduce reliance on synthetic fertilizers, promoting environmentally friendly farming. The application of CRISPR-Cas9 in agriculture is also subject to regulatory frameworks, potential unexpected consequences, and ethical considerations, all of which require careful evaluation. This review highlights these aspects, emphasizing that responsible research and development are essential for the ethical and long-term use of this technology. It attempts to illustrate the transformative capability of this technology for creating a more sustainable and food-secure future by critically assessing its possibilities and challenges.

Keywords: CRISPR-Cas9, Crop improvement, Gene editing, Green revolution, Sustainable agriculture

INTRODUCTION

Population forecasts indicate that the global population will reach approximately 10 billion by 2050 (Hunter *et al*., 2017), with some projections suggesting around 9.8 billion, alongside a potential 20% decline in food production due to the impacts of climate change (Naglaa *et al*., 2023). In 2023 alone, an estimated 713-757 mil-

lion people faced hunger globally (UN, 2024). During this period, available farmland and freshwater resources are expected to decline, while overall food consumption is projected to increase by 25% to 70%, potentially surpassing current production levels. This presents a significant challenge in feeding a rapidly expanding population, especially in the context of climate change. Therefore, it is vital to enhance food production and foster sustainable agricultural development. The Green revolution, which occurred between the mid-20th century and the present, significantly transformed agricultural methods, including advancements in crop breeding, irrigation, and the use of agrochemicals. Visionaries like Norman Borlaug led this movement, utilizing mechanized farming, extensive fertilizer use, and the adoption of high-yielding crop varieties to increase crop yields and reduce global hunger (Pingali, 2012). The introduction of fertilizer-responsive semi-dwarf wheat and rice cultivars during the 1950s significantly increased crop yields, particularly in South Asia and Latin America (Evenson and Gollin, 2003). The widespread adoption of these technologies led to a structural shift, improving global food security and transforming rural societies. Over the past few decades, the world's food supply has increased significantly due to the application of selective breeding techniques and advancements in agricultural operations, including the development of improved irrigation systems, the use of chemical fertilisers, and the introduction of heavy machinery (Pingali *et al*., 2012). Recent reviews have emphasized the role of CRISPR/Cas9 technology in enhancing crop yield, nutritional quality, and stress tolerance, thereby contributing to global food security (Rasheed *et al*., 2021). However, traditional breeding methods are often too slow to respond effectively to rapid environmental changes and the escalating pressures of climate change (Afzal *et al*., 2023; Ambika *et al*., 2024).

CRISPR-Cas9 is regarded as a revolutionary tool for editing genes, marking a substantial breakthrough in our comprehension and manipulation of genomes. This system, lauded for its high accuracy, versatility, and ease of design, is pivotal for developing climate-smart crops (Kaur *et al*., 2025) This method, which was modified from a bacterial immune system, offers previously unheard-of opportunities for precisely targeting and modifying particular DNA sequences, so modifying an organism's genetic composition. Compared to earlier genome editing tools, such as ZFNs and TALENs, CRISPR/Cas systems are more efficient and cost-effective, thereby accelerating their application in crop improvement (Ghoshal, 2024). CRISPR-Cas9 has enormous potential in plant breeding to address current issues and advance agriculture toward a more productive and sustainable future (Hsu *et al*., 2020). Recent analyses have underscored its transformative power in developing climate-resilient crops and enhancing future food security (Ahmad, 2023; Verma *et al*., 2023; Ahmad *et al*., 2021).

The framework of CRISPR-Cas9 complex technology relies on its simplicity and effectiveness, leveraging two key components: the single-guide RNA (sgRNA), which precisely directs the Cas9 protein to its target on the DNA, and the Cas9 protein itself, which functions as an enzyme and molecular scissors. Recent advancements, including base editing and prime editing, have further refined the precision and scope of genome editing, enabling more complex genetic enhancements with fewer off-target effects (Naeem and Alkhnbashi, 2023; Saber Sichani *et al*., 2023). Prime editing, for instance, combines Cas9 with reverse transcriptase, offering the potential to correct a vast majority of known pathogenic genetic variants by directly rewriting DNA sequences (Chen and Liu, 2023). With such extraordinary precision, researchers can introduce desired alterations, including adding new genes, removing undesired ones, or altering or repairing particular gene. CRISPR-Cas9 presents numerous fascinating opportunities in the context of plant breeding. Crops can be genetically modified to display desired characteristics, such as greater resistance to diseases and pests (Tang *et al*., 2023a; Vu *et al*., 2023), a reduced need for chemical pesticides, and encouragement of environmental sustainability. (Li *et al.,* 2020; Kumar *et al.,* 2020), enhanced resilience to abiotic stressors like heat (Younas *et al*., 2024; Yadav *et al*., 2023), salinity (Feng *et al*., 2023), and drought (Abdul Rahim *et al*., 2024), is crucial for preserving food in light of food sustainability, security, and climate change (Kumar *et al*., 2023; Shendekar *et al*., 2024). In the Indian context, CRISPR/Cas9 technology has been recognized as a powerful tool to combat agricultural threats, including pest and disease pressures, and to accelerate the development of resilient crop varieties (Marotkar *et al*., 2020). CRISPR/Cas9-based genome editing has emerged as a powerful strategy to enhance yield, disease resistance, and stress tolerance in crops, as highlighted by recent reviews (Saravanan *et al*., 2021).

Table 1 provides a comprehensive overview of the applications of CRISPR-Cas9 in agricultural advancement, including the enhancement of nutritional content and biofortification of crops with vital vitamins and minerals, such as increasing iron content in rice (Chen *et al*., 2024; Li *et al*., 2023), which can help combat malnutrition. According to Nasir *et al*. (2021) and Ainouz *et al*. (2022), Efficient nitrogen fixation can potentially reduce the need for synthetic fertilizers and mitigate environmental pollution. (Garg *et al.,* 2020; Santillán *et al.,* 2022)

The current challenge is to enhance or develop new technologies and solutions to boost crop yields, considering the importance of ensuring sustainable agricultural yields. The detailed understanding of CRISPR/Cas

mechanisms underscores its effectiveness in addressing global challenges such as food insecurity and the impacts of climate change (Raza *et al*., 2024). These are merely a glimpse of CRISPR-Cas9's potential in agriculture. The details of the Potential benefits and limitations of CRISPR-cas9 are provided in detail in Fig. 2. There is an urgent call for innovative solutions, and CRISPR technology is increasingly recognized for its potential to deliver these advancements, particularly in enhancing climate resilience and promoting sustainability in agriculture worldwide (Diplomatist, 2024). This study examines the potential of genome editing, specifically the CRISPR/Cas9 system, to enhance crop productivity and mitigate the adverse effects of environmental stress.

Beginning in the mid-20th century, the Green Revolution significantly increased food production. However, significant challenges, such as environmental degradation from intensive farming, emerging micronutrient deficiencies in staple crops, and the overarching need for more sustainable and resilient agricultural practices, have become increasingly apparent (FAO, 2023; Pingali, 2023). In this context, CRISPR-Cas9, a groundbreaking genome editing technique, is now widely recognized for its transformative potential to address these complex issues and contribute signifi-

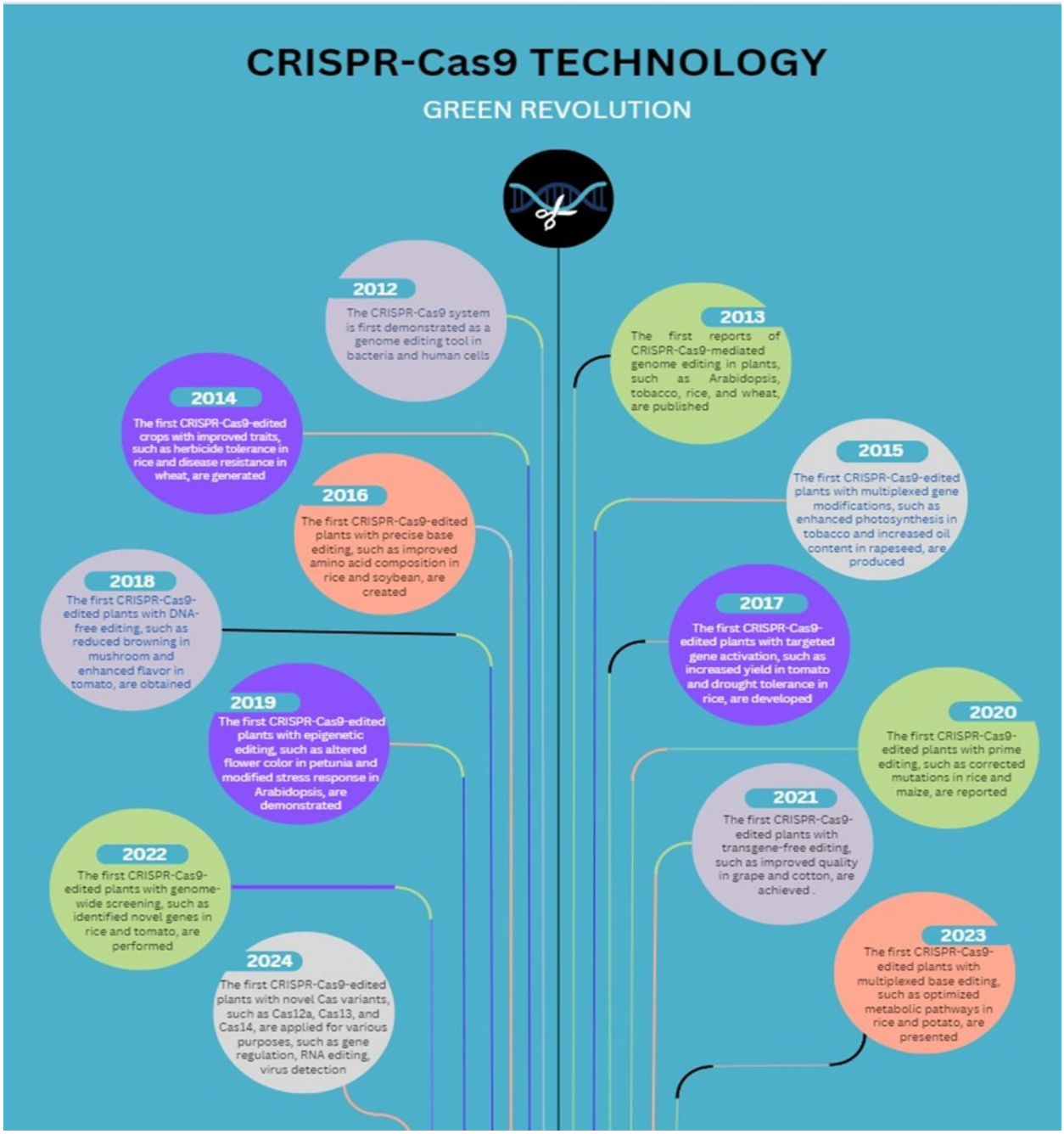

Fig. 1. *A timeline of revolutionary genome editing CRISPR-cas9 technology in agriculture*

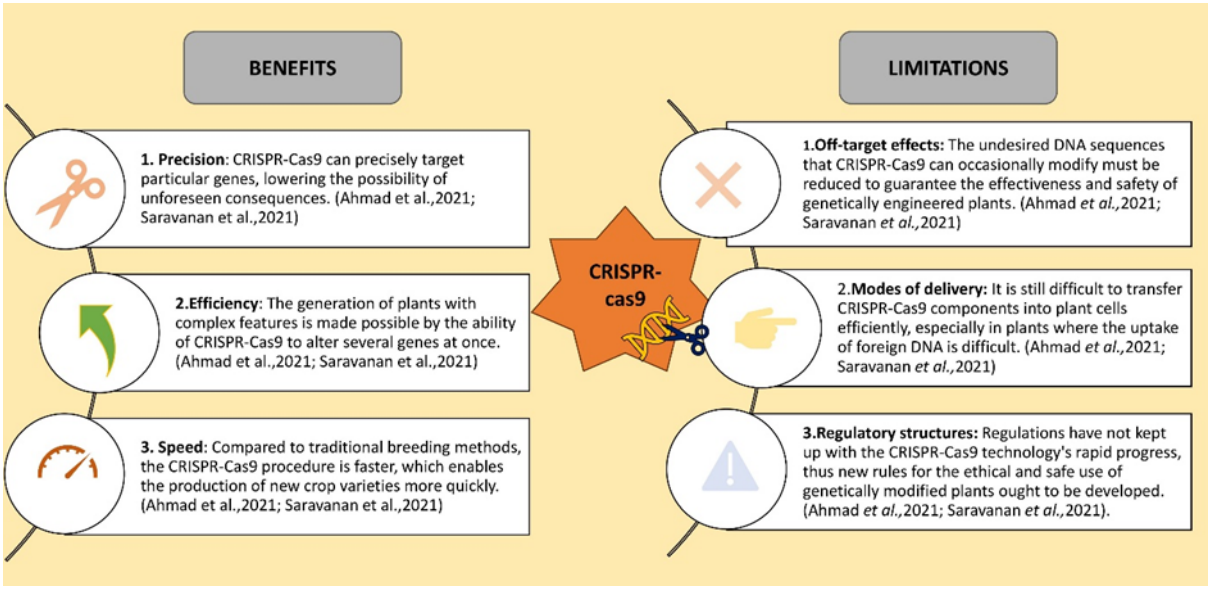

Fig. 2. *Potential benefits and limitations of CRISPR-cas9 with detailed explanation*

cantly to a new phase of sustainable agriculture, often referred to as a "Second Green Revolution" (Haji and Natesan, 2023; OECD, 2024). Compared to conventional breeding techniques, which are often time-consuming and less precise, this technology has been successfully implemented in numerous crop plants, including key staples like wheat, rice, and maize. Recent advancements have demonstrated its efficacy in developing crops with enhanced characteristics, such as improved tolerance to pests and diseases (Kaur *et al*., 2025; Ndudzo *et al*., 2024), and greater resilience to diverse environmental challenges, including drought and salinity (Verma *et al*., 2023; Kumar *et al*., 2023; Callaway, 2020).

A new era of sustainable agriculture and unlock new Green Revolution potential can be realized through the careful and ethical application of CRISPR-Cas9 technology. The potential of CRISPR-Cas9 in enhancing disease and stress resistance, yield, and crop quality is detailed in Fig. 3. By addressing the shortcomings of the earlier Green Revolution, this emerging phase could ensure food security while safeguarding the environment for future generations. This investigation highlights the promising opportunities and critical considerations associated with CRISPR-Cas9, paving the way toward a more equitable and sustainable agricultural future.

Mechanism of CRISPR-Cas9

CRISPR/Cas9 consists of the “Cas protein-encoding gene”, “CRISPR locus”, at the 3' end and Tracr RNA at the 5' end (Fig 4. a). The CRISPR locus contains a repeat sequence of 23 to 50 base pairs and the spacer sequence. The CRISPR/Cas9 system functions through the collaborative action of two primary components: sgRNA and Cas9. DNA endonuclease Cas9 (Fig. 4.b) and is derived from various bacteria, including *Streptococcus thermophilus, Brevibacillus laterosporus, Staphylococcus aureus,* and *Streptococcus pyogenes*. Interestingly, because *Streptococcus pyogenes* is so widely applicable, it is frequently used for Cas9 isolation (Karvelis *et al*., 2015). The Cas9 enzyme comprises two essential domains: the HNH and RucV-like. The RucV-like domain is responsible for cleaving the non-target strand of the double-stranded DNA, while the HNH domain facilitates the cleavage of the complementary strand of crRNA (Ran *et al*., 2015; Anzalone *et al*., 2019). The synthetic sgRNA, approximately 100 nucleotides in length, plays a crucial role in guiding Cas9 to its target site.

At the 5'-end of the sgRNA, a protospacer adjacent motif (PAM) sequence typically follows the consensus NGG pattern, where N represents any nucleotide and G signifies guanine. Additionally, a 20-nucleotide sequence serves as a guide to identify the target sequence (Geng *et al*., 2016; Steinert *et al*., 2015). Furthermore, the anchoring of the targeted sequence is achieved through the guide sequence, which is facilitated by the sgRNA, consisting of a loop structure at the 3' end, thereby creating a compound with Cas9. This complex then causes the double-stranded DNA to cleave, creating a double-strand break (DSB) at the specified location (Jacobs *et al*., 2015). After making a double-strand break (DSB), the cell initiates DNA repair activities, primarily homology-directed repair (HDR) and non-homologous end-joining (NHEJ). In most instances, the primary repair route is NHEJ, which provides a simple means to create mismatches and cause deletions and insertions (indels) in genes, which leads to gene knockout. Conversely, HDR, facilitated by the presence of an oligo template, enables precise gene replacement or the introduction of non-native DNA (Chang *et al.,* 2015; Zang *et al*., 2019) (Fig. 4c).

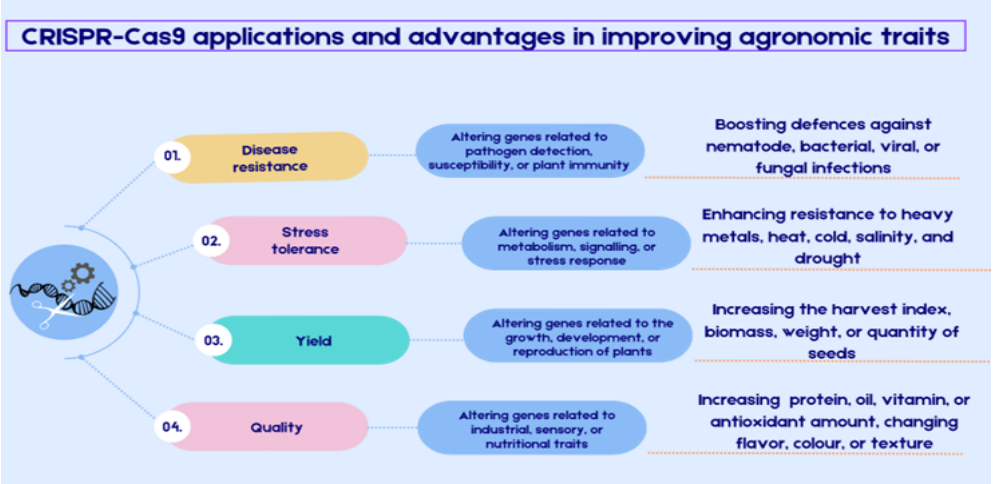

Fig. 3. *Applications and advantages of CRISPR-Cas9 in improving agronomic traits*

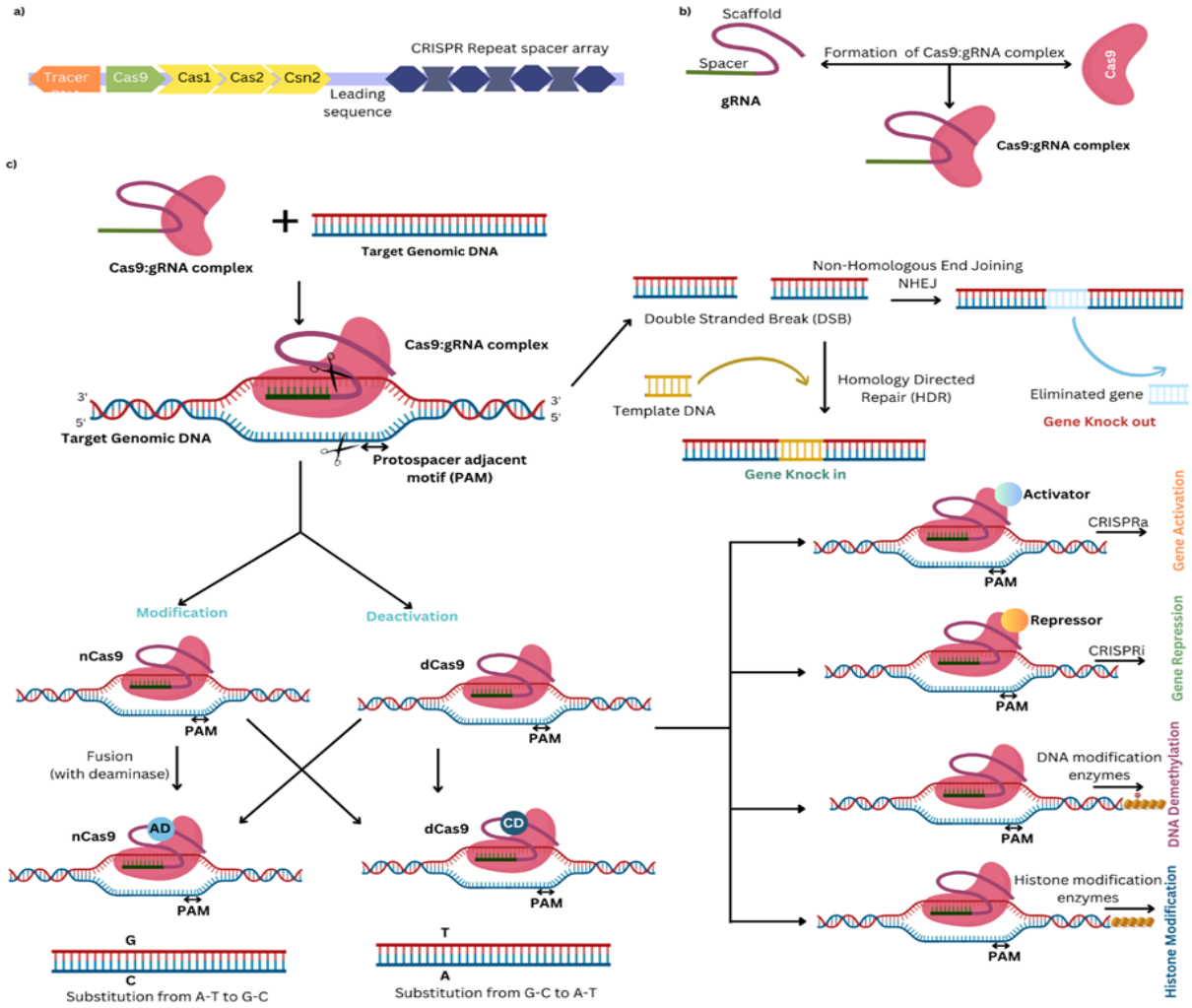

Fig. 4. *CRISPR-Cas9 mechanism of action (**a)** The CRISPR-Cas9 Structure including, CRISPR locus, at 3' end Cas protein-encoding gene, and Tracr RNA at 5' end. There are two sequences in the CRISPR locus: a spacer sequence and a repeat sequence with 23 to 50 base pairs. **b)** Formation of Cas9:gRNA complex. The gRNA contains the scaffold and spacer sequence. Cas9 consists of two essential domains: the RucV-like domain and the HNH domain; **c)** CRISPR-Cas9 acts as a molecular scissor and aids in the precision modification of genes. The genes can be edited using the knock-in or knockout methods by adding or eliminating a gene of interest (target DNA) and modifying genetic expression through DNA methylation and histone modification to achieve the desired responses)*

Tools for editing genes using CRISPR-Cas9

CRISPR/Cas9 has emerged as one of the most versatile tools for modifying specific genes across different genomes in recent years. Its continuous evolution has solidified its status as a cornerstone of modern molecular biology (Ahmad, 2023) The advent of multiple CRISPR/Cas9-based techniques (like gene knockout, gene knock-in, regulation of genes, editing of DNA bases, and prime editing) has allowed us to alter genes in a number of ways, offering unprecedented precision and flexibility in genome engineering (Gaillochet *et al*., 2021; Ansori *et al*., 2023).

Gene knockout

Gene knockout approaches help to analyze and validate gene functions and the accompanying adjustments to biological characteristics. For instance, plant gene editing can be achieved through the construction and transfer of CRISPR/Cas9 gene knockout vectors; it is possible to create target-gene knockout mutants following screening for one or two generations. The development of efficient multiplex gene knockout systems, allowing simultaneous disruption of several genes, has been a significant focus, further accelerating functional genomics studies in plants (Saini *et al*., 2023). These multiplex constructs assembly efficiency and predictability (Ahmad *et al*., 2023). Knocking down one target gene at a time or multiple target genes simultaneously is possible. Golden Gate, Gibson, or total polymer methods can insert multiple sgRNAs into a binary vector, with recent refinements aimed at improving the process (Shaheen *et al*., 2023). Targeting different locations within a single gene can enhance the effectiveness of gene editing. (Rouatbi i.,2022; Ma *et al.,*2015; Shi *et al.,* 2018)

Gene knock-in

Utilizing gene knock-in techniques, a foreign DNA fragment is inserted into a specific genomic region following Cas cleavage of the DNA. After a precisely specified Double-Strand Break (DSB) is inserted into the genome, two primary pathways, Homology-Directed Repair (HDR) and Non-Homologous End Joining (NHEJ), are carried out for repairing DNA, depending on the attributes of the donor (vector) and the stage of the cell cycle. (Bazaz *et al.,* 2022). Improving the efficiency of HDR, which enables precise template-mediated insertions, remains a key challenge; however, novel strategies, including the use of HDR enhancers and modified Cas proteins, are continually being explored (Huang *et al*., 2023; Pacesa *et al*., 2024). The cellular DNA break repair pathways provide evidence that they could increase the usefulness and effectiveness of techniques for inserting genes. One of the two main approaches used in CRISPR-based gene knock-in techniques is homology-independent targeted insertion events (NHEJ-based) or homology-dependent targeted insertion events (HDR-based). (Bazaz *et al.,* 2022). NHEJ-based methods, although often more efficient, can result in variable insertion patterns; however, recent efforts focus on controlling the integration process with greater precision (Prado *et al*., 2024).

Base editing

Single-base editors (SBEs) and double-base editors (DBEs) have greatly advanced base editing technology. Initially, SBEs were introduced as adenine base editors (ABEs) and cytosine base editors (CBEs), which permit accurate transitions from A•T to G•C and from C•G to T•A, respectively (Nishida *et al*., 2016; Komor *et al*., 2016). SBEs, however, had little use in site saturation mutagenesis. The CBE system comprises Nickase Cas9 (nCas9), cytosine deaminase, uracil glycosylase inhibitor (UGI), and sgRNA. Within the target window, cytosine deaminase converts C to U, and nCas9 facilitates cleavage of the non-target strand, resulting in a C -to-T mutation during DNA replication (Komor *et al*., 2016; Doman *et al*., 2020). In contrast, the ABE system comprises sgRNA, nCas9, and adenine deaminase. During DNA replication, adenine deaminase catalyzes the conversion of adenine (A) to inosine (I) within the target window. It is then recognized as G, leading to an A-to-G transition (Gaudelli *et al*., 2017). Recent advancements have expanded the repertoire of base editors, including C-to-G base editors (CGBEs) and editors with broader targeting ranges or reduced off-target effects (Koblan *et al*., 2023b; Ebrahimi *et al*., 2024). For example, saturated targeted endogenous mutagenesis editors offer greater adaptability by allowing C and A to be converted to G at target sites, thereby significantly expanding the range of base mutations that can be produced (Li *et al*., 2020). The development of dual-base editors capable of mediating multiple types of base conversions simultaneously further enhances their utility (Ijaz *et al*., 2023).

Prime editing

David Liu introduced the precise gene-editing technique known as prime editing (PE) in 2019. It doesn't require donor DNA or DSBs. It doesn't require donor DNA or DSBs for many types of edits (Basu *et al*., 2023). Modified from CRISPR-Cas9, PE allows all 12 types of base-to-base conversions, as well as small insertions and deletions. The Moloney murine leukemia virus (M-MLV) reverse transcriptase fused to nCas9 (H840A) and a prime editing guide RNA (pegRNA) constitute the core. A primer-binding site (PBS) and a reverse transcription template (RTT) are carried by the modified gRNA or pegRNA. Guided by pegRNA, nCas9 (H840A) induces a single-strand break in the target DNA. (Huang *et al*., 2023) The 3' end of the nicked strand then hybridizes to the PBS on the pegRNA, and

the RTT is utilized by the M-MLV reverse transcriptase to synthesize a new DNA strand containing the desired edit. This initiates automated repair, integrating base substitutions or small indels into the DNA. Because PE may alter any base without DSBs for many applications, it provides a secure and flexible gene-editing method. The Plant Prime Editor was created in 2020 by Gao Caixia's group, enabling a variety of single-base substitutions in wheat and rice (Lin *et al*., 2020). Since then, significant efforts have been made to improve PE efficiency in plants, including optimizing pegRNA design, developing new PE variants (e.g., PE4, PE5, twinPE), and enhancing the expression of PE components (Singh *et al*., 2023; Chen *et al*., 2024; Zong *et al*., 2022). A robust CRISPR/Cas9 system adaptable to both monocot and dicot species has been established, facilitating efficient multiplex editing for functional studies and crop improvement (Ma *et al*., 2015). The application of prime editing for precise gene modification in various crop species continues to expand, demonstrating its potential for agricultural biotechnology (Tuncel *et al*., 2025).

CRISPR-Cas9 scientific reports in several plants

Rice (*Oryza sativa*)

In their study, Zhou *et al*. (2019) aimed to enhance both yield and quality in rice by developing novel elite rice varieties. They identified three promising rice strains—J809, L237, and CNXJ—and focused on editing three key Quantitative Trait Loci (QTLs) related to yield. Specifically, they targeted the genes OsGS3, OsGW2, and OsGn1a, which are known for their roles in controlling grain size, width, weight, and number. Employing CRISPR–Cas9 technology, they simultaneously mutated these three genes using the Cas9 expression backbone vector pZHY988. The triple mutants exhibited an improvement in grain output across various genetic backgrounds. Through meticulous analysis of single, double, and triple mutants, the researchers observed varying impacts of these mutations on crucial variables, such as grain yield per panicle and thousand-grain weight (Zhou *et al*., 2019).

CRISPR/Cas9 editing of rice starch branching enzyme I (SBEI) and IIb (SBEIIb) was done by Sun *et al*., in 2017, yielding homozygous or bi-allelic mutants with indels at frequencies of 26.7-40% in the T0 generation. Mutations were transmitted stably to T1, with transgene -free plants carrying only frame-shifted mutations. While sbeI mutants showed no significant changes, sbeII mutants exhibited increased long chains in amylopectin, resulting in higher amylose content (up to 25.0%) and resistant starch (up to 9.8%). This study demonstrates the efficacy of CRISPR/Cas9 in enhancing rice's nutritional properties by modifying SBEIIb, paving the way for the development of high-amylose rice. (Sun *et al*.,2017)

Abe *et al*. (2018) conducted a study to improve the fatty acid composition of rice bran oil (RBO) by targeting the fatty acid desaturase 2 (FAD2) gene, which plays a crucial role in converting oleic acid to linoleic acid. The researchers used CRISPR/Cas9 system to successfully disrupt the OsFAD2-1 gene in rice, creating homozygous knockout plants. Remarkably, the amount of oleic acid in the mutant plants grew to more than double compared to the wild type, whereas linoleic acid levels dropped substantially to untraceable amounts in the brown rice seeds. This accomplishment illustrates the ability of CRISPR-Cas9-mediated mutagenesis to significantly increase the fatty acid composition of rice, demonstrating its effectiveness in tissue-specific trait improvement based on dominant gene expression (Abe *et al*., 2018). CRISPR/Cas9-mediated editing of OsDST gene in the indica rice cultivar MTU1010 resulted in improved drought and salinity tolerance, providing an efficient approach for abiotic stress management (Santosh Kumar *et al*., 2020).

Further studies in rice using CRISPR/Cas9 include further strides in enhancing disease resistance and grain quality. For example, novel strategies are being employed to develop broad-spectrum resistance against pathogens like *Magnaporthe oryzae*, the causal agent of rice blast. Recent research has demonstrated the successful editing of susceptibility genes or the modulation of defense pathways, leading to enhanced resistance without yield penalties (Yang, J. *et al*., 2023). Furthermore, efforts in 2023 and 2024 have focused on improving nutritional value beyond amylose content, such as enhancing essential amino acid levels or micronutrients like iron and zinc using precision base editing and prime editing techniques (Chen, F. *et al*., 2024). These studies highlight the ongoing refinement and application of CRISPR technology for the multifaceted improvement of rice.

Wheat (*Triticum aestivum*)

Shan *et al*. (2013) pioneered the use of CRISPR-Cas9 technology in wheat to knock out the TaMLO locus, TaPDS, and TaINOX (Shan *et al*., 2013; Upadhyay *et al*., 2013). Wang *et al*. (2014) achieved the simultaneous deletion of the three TaMLO homeoalleles to provide resistance against powdery mildew (PM) in bread wheat. Kim *et al. (2018)* presented a significant advancement in wheat genome editing, focusing on two crucial abiotic stress-responsive genes, TaDREB2 and TaERF3, in protoplasts. Additionally, they achieved a notable milestone by implementing base editing technology, exemplified by the precise replacement of a single base. (C to T) within the wheat LOX2 gene (Kim *et al*., 2018). RNA-guided genome editing has been successfully applied in wheat for targeted gene muta-

Table 1. Applications of CRISPR/Cas9 for improving crops as examples

Category	Work done on	Improvement	Editing type	Target area	Outcome	Reference
Disease resistance	Rice	*OsSWEET11* gene (sugar transporter regulation)	Knockout	*OsSWEET 11* protein	Reduced susceptibility to bacterial blight	Li *et al*. (2020)
	Wheat	*Mlo1* gene (fungal pathogen entry factor)	Knockout	*Mlo1* protein	Increased resistance to powdery mildew	Wang *et al*. (2020)
	Tomato	*SlEIX* gene (involved in pathogen perception)	Knockout	*SlEIX* protein	Elevated resistance to late-blight disease	Wang *et al*. (2018)
	Tomato	Promoter editing of endogenous defense gene (*SlNPR1*)	Knock-in	*SlNPR1* promoter region	Broader disease resistance via enhanced defense gene expression	Li *et al*. (2020b)
Stress tolerance (Earlier)	Rice	*OsPPAC2* gene (regulates stomatal closure)	Knockout	*OsPPAC2* protein	Improved drought tolerance via enhanced water retention	Xu *et al*. (2018)
	Maize	*ZmZAP10.2* gene (heat stress response regulator)	Knockout	*ZmZAP10. 2* protein	Increased heat tolerance by promoting heat shock protein production	Zhai *et al*. (2020)
	Tomato	*SlDREB2C* gene (involved in salt stress response)	Base editing	*SlDREB2C* protein	Enhanced salt tolerance through improved cellular osmoprotection	Zhang *et al*. (2020)
Climate resilience (New)	Rice	Multiplex editing of stress-responsive regulatory genes (e.g., *OsDREB* and *OsHSP* families)	Multiplex gene editing (combined knockout/ activation)	Regulatory network governing drought and heat tolerance	Stable yields under fluctuating climatic conditions; enhanced resilience to drought and high temperatures	Kaur *et al*. (2025)
Abiotic stress tolerance (New)	Wheat	Targeted knockout of a key transcription factor gene (e.g., a *TaDREB* homolog)	Knockout	Stress response signaling pathway	Improved water-use efficiency and increased survival under drought and saline conditions	Adane and Alamnie (2024)
Nutritional value	Rice	*OsPSY1* gene (controls beta-carotene biosynthesis)	Base editing	Specific nucleotides in the *OsPSY1* gene	Increased beta-carotene content (vitamin A precursor)	Li *et al*. (2017)
	Cassava	*Spudap1* gene (regulates protein storage)	Knockout	*Spudap1* protein	Elevated storage protein levels, enhancing nutritional quality	Zhang *et al*. (2022); Carluccio *et al*. (2022)
Nitrogen fixation	Legume (Soybean)	NF symbiosis 1 (*NFs1*) gene cluster	Knockout	*NFs1* gene cluster (required for nodule formation)	Engineering symbiotic nitrogen fixation with compatible bacteria	Garg *et al*. (2020)
Herbicide tolerance	Rice	Promoter editing of endogenous *ALS* gene	Knock-in	*ALS* gene promoter	Increased tolerance to ALS-herbicides, enhancing weed control efficiency	Xu *et al*. (2017)

Contd…….

Table 1. Contd…….

Improved quality	Tomato	*SlRIN* gene (ripening inhibitor)	Knockout	*SlRIN* protein	Delayed fruit ripening and extended shelf life	Li *et al*. (2019)
	Banana	*MaETR1* gene (mediates ethylene perception)	Knockout	*MaETR1* protein	Reduced ethylene sensitivity; delayed ripening and prolonged shelf life	Ramirez *et al*. (2020)
Yield optimization (New)	Soybean	Precise knock-in of favorable alleles in yield-related genes (e.g., *GmSWEET* homologs)	Precise gene editing (knock-in)	Promoter regions/ coding sequences of key yield□determining genes	Enhanced pod number, increased seed size, and improved overall biomass leading to higher yield	Kumar *et al*. (2023)

tions, laying the foundation for CRISPR-based crop improvement in cereals (Upadhyay *et al*., 2013).

Zhang *et al*. (2019) demonstrated the efficacy of an Agrobacterium-delivered CRISPR/Cas9 technology in wheat, using a single binary vector that included a wheat codon-optimized Cas9 and guide RNA cassette. This approach enabled the creation of 68 edited mutants for important grain-regulatory genes in T0, T1, and T2 gen plants, with a mean edit rate of 10%, and no off-target mutations in the maximum active Cas9 plants. Notably, Homozygous mutations can be acquired in a single generation from a large population, with deletions exceeding 10 base pairs being the primary mutation type in wheat. Homozygous mutants with an 1160 base pair deletion in TaCKX2-D1 showed a substantial increase in grain number per spikelet. This study highlights the potential of Agrobacterium-delivered CRISPR-Cas9 as a promising method for efficient genome editing in wheat, reducing the need for a large T0 transgenic plant population and offering novel mutation opportunities across generations (Zhang *et al*., 2019). Cai *et al*. **(2018)** achieved increased grain weight and yield in wheat by overexpressing the TaGW2 gene, which regulates grain weight. CRISPR/Cas9-mediated knockout of the Ms1 gene has enabled the development of male-sterile hexaploid wheat lines, offering a strategy for rapid hybrid seed production (Okada *et al*., 2019)

Gupta *et al*., (2023) studied the role of TaSPL13 genes in wheat to improve grain production and agronomic traits. They used CRISPR-Cas9 to change the microRNA156 recognition elements (MRE) of TaSPL13, resulting in 12 mutations and a two-fold rise in TaSPL13 transcript levels. The mutants had shorter blooming times, fewer tillers, and taller plants, but larger and more grains. These findings suggest that TaSPL13 mutants have the potential to increase wheat yield by improving grain properties and plant architecture. This study demonstrates the use of CRISPR-Cas9 in wheat breeding to increase agronomic properties. (Gupta *et al*., 2023).

Wheat continues to expand the frontiers of gene editing for enhanced resilience and productivity. The use of advanced CRISPR tools, such as base editing and prime editing, to introduce precise modifications for traits like herbicide tolerance and improved dough quality, minimizing off-target effects, is highlighted (Zhou *et al*., 2023). Furthermore, significant efforts are directed towards engineering durable resistance against devastating fungal diseases, such as rusts and Fusarium head blight, by targeting multiple genes or regulatory elements simultaneously. This strategy is made more feasible by refined multiplex editing systems (Dong and Fan, 2024). These ongoing developments are crucial for adapting wheat to changing environmental conditions and meeting global food demands.

Tomato *(Solanum lycopersicum)*

In an investigation conducted by Karkute *et al*. (2017), phytoene desaturase (SlPDS), phytochrome interacting factor (SlPIF4), and Phytoene synthase (PSY1) are examples of genes involved in carotenoid biosynthesis. The CRISPR/Cas9 technology was utilized to modify these genes. Fruits have undergone changes in size, colour, and nutritional value as a result of these mutations. To investigate the functional validation of developmental genes in tomatoes, such as SELF PRUNING 5G (SP5G) and SELF PRUNING (SP), CRISPR/Cas9 has been employed to target these genes. Both genes were mutated, producing compact plants that flowered early. Tomato fruits that can develop without fertilization, known as parthenocarpic tomatoes, have been created via CRISPR/Cas9 technology. This was accomplished by deleting the Slagamous-like 6 (SlAGL6) gene, which enabled the plants to generate parthenocarpic fruits in the face of heat stress—a highly sought-after trait in the processing sector. (Karkute *et al*.,2017).

Santillán Martinez *et al*. (2020) investigated the impact of complete knockout of the PMR4 gene on resistance to the PM pathogen Oidium neolycopersici (On) by creating mutants of the susceptibility (S) gene PMR4 in tomatoes using CRISPR/Cas9. In comparison to plants of the wild type, the study found that five distinct mutation events—including deletions, insertions, and inversions—all showed decreased susceptibility to On. Histological studies in the pmr4 mutants showed a higher frequency of hypersensitive response-like cell death at fungal infection sites, suggesting that CRISPR/Cas9 is a promising method for investigating and defining S-genes.

Transgene-free genome-edited tomatoes, including the non-transgenic 'Tomelo' variety, have been created using CRISPR/Cas9. This technique removes foreign DNA sequences from the genome of the tomato. This variety was created in just 9.5 months, starting with the DNA transformation phase and ending with the recovery of second-generation transgene-free plants. It is resistant to PM. (Nekrasov *et al.,2017;* Ito *et al*., 2015*)*

The identification of a receptor for the fungal elicitor ethylene-inducing xylanase (EIX) as part of a resistance-like gene family in tomato illustrates the potential of molecular approaches to enhance pathogen resistance (Ron and Avni, 2004). According to researchers (Barman *et al*., 2019; Li *et al*., 2018a and Li *et al*., 2018b), lycopene-enriched tomatoes (*Solanum lycopersicum*) are produced by simultaneously editing genes involved in lycopene biosynthesis and manipulating genes in the GABA shunt pathway to increase the amount of gamma-aminobutyric acid (GABA) in tomatoes.

Current research on tomatoes emphasizes the development of varieties with enhanced nutritional profiles and robust stress tolerance. For instance, researchers are utilizing CRISPR/Cas9 to fine-tune metabolic pathways for increased accumulation of beneficial compounds, such as vitamins and antioxidants, extending beyond lycopene (Tiwari *et al*., 2023). Additionally, new studies are demonstrating the potential of gene editing to confer resistance to emerging viral threats and to improve tolerance to complex abiotic stresses, such as combined heat and drought, which are becoming increasingly prevalent due to climate change (Shawky *et al*., 2024). Genome editing of SlHyPRP1 and SlDEA1 genes in tomato using CRISPR/Cas9 conferred tolerance to multiple stress factors, underlining the utility of this tool in stress-resilient crop breeding (Saikia *et al*., 2024). These advancements highlight CRISPR's role in developing consumer-friendly and climate-resilient tomato varieties.

Potato (*Solanum tuberosum*)

According to Johansen *et al*. (2019), CRISPR/Cas9 was utilized to target the SS6 gene in potatoes, resulting in deletions and altered starch production with promising industrial applications. The researchers demonstrated that utilizing endogenous plant-specific U6 promoters enhanced CRISPR/Cas9 editing efficiency, increasing sgRNA levels and improving editing frequencies. Specifically, their work demonstrated high effectiveness, achieving complete allelic CRISPR/Cas9 genetic editing in tetraploid potatoes, with editing efficiencies of up to 35% at the ex-plant level. This approach holds significance for enhancing starch quality in potatoes and presents a valuable strategy for gene editing in polyploid crops, offering insights for improving various traits in agricultural applications.

Tussipkan *et al*. (2021) employed CRISPR/Cas9 to target the St16DOX gene in tetraploid potatoes, aiming to enhance potato-tuber quality by reducing anti-nutritious substances, such as steroidal glycoalkaloids and acrylamide. Through this approach, they successfully induced multiple mutations in the St16DOX gene, eliminating wild-type sequences and demonstrating the potential for improving potato quality through gene editing. The effectiveness of CRISPR/Cas9 in modifying biosynthetic pathways to enhance the nutritional characteristics of potatoes has been shown (Tussipkan *et al*., 2021).

Ly ***et al***. (2023) demonstrated how to target the S-RNase gene with CRISPR/Cas9 to overcome self-incompatibility in diploid potatoes. This method produced self-compatible diploid potato lines, which presents a viable plan for the productive creation of inbred/F1 hybrid cultivars in potatoes.The study by Kieu *et al*. (2021) demonstrated the successful application of CRISPR/Cas9 to mutate and screen seven potential S-genes, including two DMR6 potato homologs, to produce potato plants with enhanced resistance to late blight. Potato plants with functional knockouts of StDND1, StCHL1, and DMG400000582 (StDMR6-1) exhibited enhanced resistance to late blight, suggesting that this strategy may be effective in enhancing potato disease resistance. Genome editing protocols using CRISPR/Cas9 have also been established in potato, enabling precise modifications despite the challenges posed by its vegetative propagation system (Nadakuduti *et al*., 2019).

Recent (2023-2024) CRISPR applications in potato are further refining strategies for disease resistance and quality improvement**.** For instance, studies are focusing on creating durable, broad-spectrum resistance to late blight by stacking edited genes or fine-tuning the expression of defence-related genes, thereby moving beyond single-gene knockouts (Lukasiewicz, 2023). Additionally, there is a growing emphasis on reducing cold-induced sweetening in tubers, a major concern for the processing industry, by precisely editing genes involved in sugar metabolism, thereby minimizing

acrylamide formation during frying (Egorova *et al*., 2024). These targeted modifications demonstrate the increasing sophistication of CRISPR tools in addressing specific challenges in potato cultivation and processing.

Maize (*Zea mays*)

Qi *et al*. (2016) created a tRNA-processing system-based method for CRISPR/Cas9 multiplex gene editing in maize. This method improved maize mutagenesis efficiency and expanded the number of targeted sites. The authors created three gRNAs and three multiple tRNA-gRNA units for simplex and multiplex editing, respectively. The outcomes demonstrated that this technique improved maize mutagenesis efficiency in addition to increasing the number of targeted sites.

CRISPR-Cas9 has also been utilized to create numerous independent maize target lines, each of which has a single SSILP in one of the target genes, thereby creating complex trait loci and trait stacking. Targeted insertion events have been recovered using this method in 93% of the investigated sites, demonstrating its durability and potential to enhance maize breeding and genetic research (Gao *et al*., 2020).

Chilcoat *et al*. (2017) conducted a study investigating the impact of CRISPR-Cas9 editing on maize plants that go off-target. The work carefully evaluated the off-target activity of CRISPR-Cas9 editing by combining genome-wide biochemical off-target detection with computational prediction. To minimize off-target effects and maximize on-target efficiency, a systematic evaluation is necessary to optimize the precision and accuracy of CRISPR-Cas9 editing in maize. CRISPR-Cas9 editing has the potential to aid crop improvement by providing insights into the creation of complex trait loci and trait stacking in maize. This work emphasizes the importance of carefully assessing off-target activity in CRISPR-Cas9 editing to guarantee its secure and efficient use in crop improvement projects. (Chilcoat *et al*., 2017)

Recent research on maize leveraging CRISPR/Cas9 is heavily focused on enhancing climate resilience and boosting yield potential. For instance, studies have demonstrated the successful application of base and prime editing to modify key genes involved in drought tolerance, leading to improved water use efficiency and sustained performance under water-scarce conditions (Li *et al*., 2023). Furthermore, significant progress is being made in engineering nitrogen use efficiency by editing genes related to nitrate uptake, assimilation, and signalling pathways, which could reduce reliance on synthetic fertilizers and promote more sustainable agricultural practices (Zhang *et al*., 2024). These advancements highlight CRISPR's pivotal role in developing maize varieties that are better suited for future farming systems.

Oilseed rape (*Brassica napus*)

Okuzaki *et al*. (2018) carried out work targeting oleic acid desaturation in *Brassica napus* cv. Westar modified the fatty acid desaturase 2 gene (FAD2) using CRISPR/Cas9 technology. For BnaA.FAD2.a (FAD2_Aa), two guide RNAs were created, and three of the twenty-two regenerated shoots had mutant alleles. After further investigation, two fully grown plants (Aa1#13 and Aa2#2) with mutant alleles were found. These plants showed a 4-bp deletion in the fad2_Aa allele. Backcross progenies (BC1) in the Aa1#13 line inherited this loss. The fad2_Aa allele was then chosen for transgene-free plants from BC1 progenies, and homozygous fad2_Aa plants were created by self-cross (BC1S1). In comparison to wild-type seeds, fatty acid composition analysis showed a substantial increase in oleic acid content.

Recent advances (2023-2024) in CRISPR/Cas9 applications for oilseed rape are concentrating on enhancing oil quality, yield, and stress tolerance traits. Researchers have successfully utilized multiplex CRISPR systems to simultaneously edit multiple FAD2 homoeologs, resulting in ultra-high oleic acid content and improved oil stability (Ali *et al*., 2023). Moreover, efforts are underway to improve resistance to significant diseases, such as Sclerotinia stem rot and blackleg, by targeting susceptibility factors or bolstering plant defence mechanisms, with promising results reported in recent publications (Bhat *et al*., 2024). These studies are vital for improving the economic and agronomic value of oilseed rape.

Legume (soybean)

Jacobs *et al*. (2015) proved that CRISPR/Cas9 is a useful means for editing the genomic sequence in soybean, as evidenced by the targeted DNA mutations found in ninety-five percent of hairy root transgenic events; 8 out of 9 targeting vectors had bi-allelic mutations, with minor deletions being the most occurring mutation; homoeologous genes were successfully targeted; somatic embryo cultures were altered to produce plants with heritable mutations; and a novel cloning strategy and vector system were created.

Garg *et al*. (2020) investigated the use of CRISPR-Cas9 to enhance legume nitrogen fixation, with a particular emphasis on soybeans. The NFY1A and NFYB genes were their targets. These genes are essential for synthesizing nodulation factors, which are chemical cues released by plants to interact and recruit suitable rhizobia strains.

Current CRISPR research in soybeans is actively pursuing avenues to enhance nutritional value, particularly protein content and composition, and to bolster resistance against biotic and abiotic stresses. For instance, gene editing strategies are being refined to en-

hance the accumulation of essential amino acids and reduce anti-nutritional factors in soybean seeds, thereby improving their quality for both food and feed (Yao *et al*., 2023). Furthermore, recent studies have highlighted significant progress in developing soybean varieties with enhanced tolerance to major pests, such as the soybean cyst nematode, and diseases, including soybean rust, through the precise modification of susceptibility or resistance genes (Freitas-Alves *et al*., 2024). These efforts are critical for sustainable soybean production.

Cotton (*Gossypium herbaceum*)

To improve CRISPR/Cas9 efficiency in cotton, Long *et al*. (2018) developed a transient expression system for testing and validating CRISPR/Cas9 cassettes. They significantly enhanced the efficacy of mutagenesis by fine-tuning the cotton CRISPR/Cas9 system using an indigenous GhU6 promoter, which increases sgRNA production levels compared to the Arabidopsis AtU6-29 promoter. This novel approach addresses the challenges posed by the lengthy and complex genetic transformation process in cotton, while also streamlining the identification of efficient CRISPR/Cas9 cassettes prior to stable transformation. They emphasizes how crucial it is to maximize the efficacy of CRISPR/Cas9-mediated mutations in cotton, providing a viable approach to targeted mutagenesis and crop improvement in this essential crop.

According to the research conducted by Khan *et al*. (2023), CRISPR/Cas enables the enhancement of cotton's resistance to stress, modification of gene expression, and stacking of genes for desired traits. The text highlights the potential of CRISPR/Cas9 to improve cotton quality and addresses regulatory issues related to genetically modified organisms (GMOs). The effective use of CRISPR/Cas9 to target genes important in stress resistance and fiber formation in cotton using gene editing. It discusses the potential of using genome editing to enhance cotton and the urgent need to develop a functional CRISPR/Cas9 technology for cotton (Khan *et al*., 2023).

Gao *et al*. (2017) created a quick and effective transient expression system technique for validating sgRNAs in cotton. The target sites for GhPDS, GhCLA1, and GhEF1 were verified, and the type of CRISPR/Cas9-induced mutations, which were mostly deletions (-64%), was examined. This demonstrated that the CRISPR/Cas9 system can successfully induce mutations in homologous cotton genes, enabling multiple gene targeting and the elimination of gene segments. Due to interference with chloroplast biogenesis caused by the high mutation efficiency (80.6%) in the GhCLA1 gene, a strong albino phenotype was observed. This technique offers a useful tool for sgRNA validation and enhances the effectiveness of CRISPR/Cas9-mediated genome editing in cotton.

Recent breakthroughs in cotton using CRISPR/Cas9 are focusing on accelerating the development of varieties with superior fiber quality and enhanced resilience to environmental stresses. For example, researchers have reported the successful application of CRISPR to modify key genes involved in fibre elongation and strength, resulting in significant improvements in these crucial quality parameters (Kumar *et al*., 2024). Additionally, ongoing work is leveraging gene editing to improve cotton's tolerance to Verticillium wilt, a devastating disease, and to enhance drought and salt tolerance, which are critical for expanding cotton cultivation into marginal lands (Guo *et al*., 2023). These advancements are vital for the sustainability and profitability of cotton farming.

Sugarcane

Oz *et al*. (2021) showed the capable and repeatable targeting of genes in sugarcane, allowing the exact editing of several alleles together by template-mediated and homology-directed repair. Their study focused on targeting the ALS gene, responsible for herbicide tolerance, using two sgRNAs to create ds DNA cleavage near specific codons. Research confirmed the effectiveness of sgRNAs in targeting Cas9 to induce double-strand breaks (DSBs), leading to the directed substitution of nucleotides in the ALS gene. This work demonstrates the capability of CRISPR/Cas9-Mediated gene targeting for generating mutations in homologous genes in sugarcane, a significant advancement for this complex allotetraploid crop.

The study by Hussin *et al*. (2022) presents the findings of CRISPR/Cas9-based sugarcane genome editing for sustainable production, discussing the foundations of gene editing technology, specifically the CRISPR/Cas9 system, and its application in modifying the sugarcane genome for sustainable production. The study also highlighted the prospective effects of gene editing technology on the sugarcane sector, as well as its prospects and challenges in sugarcane breeding.

By modifying the genome of the plant host and selectively targeting viral genes, the CRISPR/Cas9 system has been utilized to enhance sugarcane's resistance to plant viruses. Krishna *et al*.'s (2023) assessment emphasizes the importance of CRISPR/Cas9 in enhancing disease resistance in sugarcane, highlighting its potential for creating virus-resistant cultivars. Plant viral infections may be effectively controlled by this method, which has been demonstrated to target and change viral genes.

CRISPR applications in sugarcane aim to overcome its complex polyploid genome, enhancing sugar yield, biomass characteristics, and stress resilience. For instance, advanced strategies are being developed to efficiently edit multiple alleles of target genes involved

in sucrose accumulation and fiber content, which are critical for both sugar production and bioenergy applications (Ali *et al*., 2023). Moreover, studies are emerging that demonstrate enhanced tolerance to drought and diseases, such as smut and mosaic virus, through targeted gene modifications, paving the way for more robust and productive sugarcane varieties (Kumar *et al*., 2024). These efforts are crucial for the future of the sugarcane industry. Recent advances in transgene-free CRISPR/Cas9 editing provide opportunities to develop stress-resistant sugarcane varieties, minimizing regulatory hurdles (Surya Krishna *et al*., 2023).

Banana

Wang *et al*. (2022) showed how to effectively target genes in sugarcane using CRISPR/Cas9, allowing for the precise co-editing of multiple alleles through homology-directed and template-mediated repair. Through targeted nucleotide substitutions and amino acid modifications in sugarcane plants, their work aimed to improve herbicide resistance by targeting the acetolactate synthase (ALS) gene. This study reveals that CRISPR-Cas9 may be used to accurately modify sugarcane genes, giving a potential strategy for crop modification.

Tripathi *et al*. (2022) demonstrated how CRISPR-Cas9 -based gene editing techniques can accelerate banana improvement. Resistance to bacterial illnesses can now be engineered using the CRISPR-Cas9 genome editing technique by increasing the expression of plant defence genes or removing disease-causing susceptibility genes. To inhibit the action of dsDNA viruses in plants., CRISPR-Cas9-based genome editing has been used. One example of this is the editing of the endogenous banana streak virus from the B genome of Musa spp., which presents a significant breeding challenge for bananas. Furthermore, as DMR6 is a susceptibility gene that expresses a 2-oxoglutarate Fe (II)-dependent oxygenase (2OGO), which is elevated during pathogen infection and functions as an inhibitor of plant immunity, altering the orthologue of DMR6 in bananas has demonstrated increased resistance to bacterial illness.

In banana, using CRISPR/Cas9 is particularly focused on combating devastating diseases like Fusarium wilt (Panama disease TR4) and improving fruit quality and shelf life. For example, significant progress has been reported in engineering resistance to Fusarium wilt TR4 by targeting susceptibility genes or enhancing the expression of resistance-related genes in commercially important cultivars (Hu *et al*., 2023). Additionally, research is exploring the use of CRISPR to modify ethylene biosynthesis or perception pathways to delay ripening and extend the post-harvest life of bananas, which is crucial for reducing food loss (Tripathi *et al*., 2024). These developments are critical for the sustainability of banana production worldwide.

Conclusion

New plant breeding techniques now enable researchers to introduce desirable traits more precisely and efficiently than traditional breeding methods. Among these, CRISPR/Cas9 has emerged as a key innovation, accelerating plant breeding and crop improvement programs across various species. Over the past four years, CRISPR/Cas9 technology has progressed rapidly and holds the potential to usher in a new Green Revolution by developing crop varieties capable of addressing long-standing challenges, such as enhanced environmental adaptation, photo thermosensitivity, biological nitrogen fixation, biofortification, and biofuel production. It has been used in many plant species in recent years to enhance several economically significant features, including nutritional value, multiplex editing, resistance to living and non-living stress factors, and yield. Despite this, the genome editing-based technology CRISPR-Cas9 is an essential method for obtaining "genome-edited crops," widely utilised in staple crops globally, which will help reach a world free of hunger and poverty, ultimately feeding the world's expanding human population. Moreover, a significant portion of this technology needs to be adjusted to optimise on-target efficiency, as most of the work in progress still requires refinement. One advantage of using CRISPR-Cas9-induced genome editing is that many regions can be targeted simultaneously. Multiple disease resistances are being conferred to crop plants through novel applications of this technology. Breeding efforts for a variety of crops have increased thanks to CRISPR/Cas9, which has sparked creative applications in agricultural development. Therefore, CRISPR/Cas9 is the most innovative and dependable method for revolutionising agriculture, providing a path forward for future developments in the plant genome editing system. It is recommended to focus on the various applications of genome editing tools in crop development to enhance crop production, improve nutritional value, increase resilience to biotic and abiotic stresses, enhance quality, and other economically significant features. With that, this is probably the best technique that can unlock the new green revolution by mitigating the effects seen due to the rapid use of fertilizers and pesticides in the first green revolution.

Conflict of interest

The authors declare that they have no conflict of interest.

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