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Shaping agricultural future: a comprehensive review on crispr technology applications in agriculture

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Abstract

With the increasing global demand for food, new pathways have emerged to drive the development and manipulation of crops with desired traits to ensure food security. CRISPR technology has enabled genome editing, allowing the addition or destruction of specific DNA sequences to modify a function, technology stands out as an exceptionally versatile tool. The components of the CRISPR system include the Cas9 enzyme, responsible for double-strand DNA cuts, and the guide RNA (gRNA), forming part of the spacer RNA. Through a systematic review, we searched, identifying 30 articles related to the CRISPR technique and its application in agriculture. The CRISPR-Cas9 system has been widely employed to understand transcriptional regulation, make epigenetic modifications, and microscopically visualize specific genome loci. The results support the specificity of genome editing with the CRISPR/Cas9 system, demonstrating efficiency in enhancing crop performance, enabling the generation of plants free of foreign DNA, and avoiding off-target mutations. Overall, the technique has increased productivity, water stress resistance, and weed control in various crops evaluated. It plays a pivotal role in boosting agricultural productivity, enabling the creation of crops adapted to adverse environments, and significantly enhancing food security. CRISPR/Cas9 thus represents a fundamental tool in genetic engineering, propelling significant innovations to address global agricultural challenges.

Keywords

Cas9; Cleavage; Genetic Improvement; Genome.

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Introduction

The escalating global population has heightened the demand for food, prompting critical inquiries into the quality of these products (Ikram et al., 2024). Catastrophic losses inflicted upon farmers worldwide due to climate change, stemming from global warming, underscore the pressing need for innovative solutions. Notably, issues such as corn leaf rust in the 1970s resulted in a staggering death toll due to malnutrition, emphasizing the profound impact on society (Achary, Reddy, 2021). As the demand for food continues to rise, novel pathways have emerged to propel the development and manipulation of crops endowed with specific traits (Ikram et al., 2024).

In the nascent stages of genetics, plant modification occurred through selection and breeding, predating a nuanced understanding of genes, genetic modifications, and mutagenesis (El-mounadi et al., 2020). With advancements, diverse methods of plant genetic editing have arisen to optimize cultivation, rendering it more efficient and adaptable across various species (Angon; Habiba, 2023, Santos et al., 2018). Since the 1990s, scientists have employed molecular markers to selectively breed hybrid plant strains through genetic enhancement processes (Ikram et al., 2024).

One of the latest methodologies involves genome modification through programmable endonucleases, such as zinc finger nucleases, TALENs, and CRISPR/Cas9 (Zhang et al., 2019). At the forefront of these technologies is CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats), offering the capability to edit the genome by appending or eradicating specific DNA sequences to alter functionality (Gupta et al., 2019, Pallarz et al., 2023). CRISPR/Cas9 technology stands out as an exceptionally versatile tool, enabling precise targeting of various genes guided by RNA sequences (Pallarz et al., 2023).

Additionally, this technology has become indispensable in bacterial adaptive immunity, discovered through studies with Escherichia coli in the 1980s (Kim et al., 2018). Many bacteria utilize this technique to fragment viral DNA and retain a portion of it as a mechanism for identification and protection. Initially adapted for use in medicine, this technology later found applications in agriculture (Yin et al., 2014). CRISPR/Cas9 is currently the most popular tool in genetic editing in plants, resulting in the development of rice varieties

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tolerant to salt, drought, and cold. Moreover, high-yielding maize varieties have been created using CRISPR/Cas9 (Zeng et al., 2020a), significantly contributing to increasing agricultural productivity by preventing attacks from pests and pathogens (Zhang et al., 2019).

The components of the CRISPR system include the Cas9 enzyme, responsible for double-strand DNA cuts, and the guide RNA (gRNA), forming part of the spacer RNA (Angon; Habiba, 2023). The gRNA binds to the DNA, and the planned cutting sequence guides the Cas9 enzyme to the target location in the genome. The nitrogenous bases of the gRNA correspond to the target DNA segment, allowing the gRNA to specifically bind to the target sequence without damaging other parts of the genome (Paul; Montoya, 2020).

The DNA repair pathways for Double-strand break (DSBs) within cells can be categorized into two main groups: non-homologous end joining (NHEJ) and homology-directed repair (HDR). NHEJ is the primary pathway for repairing DSBs in most cell types but often results in random deletions or insertions (indels) near the DSB (Yu et al., 2023, Angon; Habiba, 2023). HDR can accurately incorporate desired changes using a DNA template, but it is typically active only in dividing cells and is cell-cycle phase-dependent, leading to reduced efficiency (Yu et al., 2023).

The CRISPR-Cas technology is categorized into class I and class II. Class II systems are characterized by the presence of crRNA-effector complexes composed of multiple subunits, with a single protein, Cas9, fulfilling all effector complex functions (Samai et al., 2015). This differentiation highlights the diversity in CRISPR-Cas mechanisms, with cas9 as a characteristic gene coding for a multidomain protein (Makarova et al., 2015). Cas9 plays a crucial role by combining the functions of the crRNA-effector complex and performing cleavage of the target DNA. Additionally, cas9 contributes to the system's adaptation process, making it an essential element for its effectiveness (Makarova et al., 2015, Paul ;Montoya, 2020).

In conclusion, the objective of this work was to conduct a systematic literature review to better understand the use of the CRISPR methodology in genetic improvement, its applications, and the benefits related to sustainable agriculture. This comprehensive exploration serves as a foundation for appreciating the revolutionary impact of CRISPR technology on advancing agricultural practices, paving the way for a more sustainable and productive future.

Materials and methods

A systematic review was meticulously conducted, identifying 30 pertinent articles in a comprehensive search for studies centered around the CRISPR technique and its applications. The databases of choice were Medline/PubMed and Scopus, and the selected articles were in either Portuguese or English. The search terms employed were carefully curated, including: “CRISPR,” “Cas9,” “sustainable agriculture,” “cellular biology,” “genome alteration,” “cleavage,” and “genetic improvement” (Figure 1).

In the selection process, stringent inclusion criteria were applied: (I) articles offering insights into the global landscape of sustainable agriculture; (II) articles delving into studies on genetic improvement techniques; (III) articles published in reputable national and international journals; (IV) articles employing CRISPR technology; (V) original articles, with a temporal range spanning from January 2013 to January 2024. Conversely, exclusion criteria were applied to filter out: (I) articles exclusively addressing agricultural issues; (II) articles focused on genetic improvement techniques unrelated to CRISPR; and (III) literature reviews, editorials, and case studies.

An initial survey based on article titles laid the groundwork, followed by a thorough evaluation of selected studies against the specified criteria. The extracted information encompasses various facets, including study characteristics, title, publication details, and pertinent considerations. This methodological approach ensures a comprehensive and rigorous synthesis of the selected literature, setting the stage for a robust understanding of the CRISPR methodology's role in genetic improvement for sustainable agriculture.

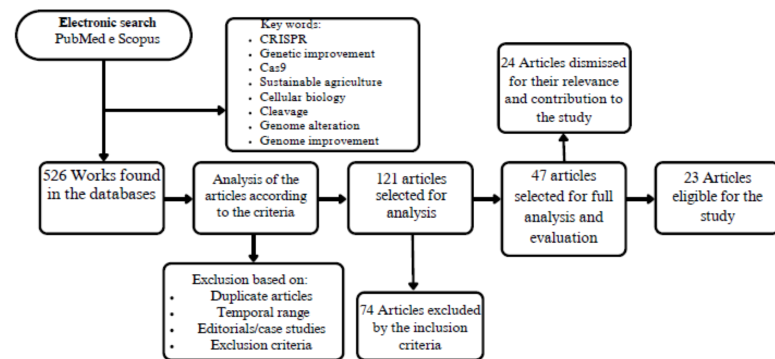

Figure 1. Flowchart of Results.

Results and discussion

CRISPR Technology

Genomic engineering has ushered in a biological revolution, introducing novel techniques for precise DNA manipulation. The capacity to edit the genome has significantly expanded our ability to intricately modify and study genes (Zhang et al., 2017). This revolutionary capability enables targeted modifications within the genome, including alterations in contexts, outcomes, and transcriptions. As a result, a suite of innovative technologies has emerged, offering the power to delete, insert, and modify DNA sequences, gene functions, and regulatory elements—among these, CRISPR stands as a pivotal player (Moreno-Mateos et al., 2015).

The genesis of CRISPR technology traces back to the defense mechanisms of bacteria, endowing them with immunity against invasive plasmids or phages. This system presents unparalleled advantages in terms of versatility, selectivity, and efficiency when juxtaposed with traditional biotechnological approaches (Samai et al., 2015). The Cas9 molecule, in association with guide RNA (gRNA) through CRISPR RNA, meticulously scans the DNA until it discovers a matching target DNA (protospacer) with the gRNA, leading to the precise cleavage of the target DNA’s double strand (see Figure 2). The sequence marked by a motif adjacent to the protospacer, known as the protospacer adjacent motif (PAM), serves as the pivotal determinant for binding specificity (Lin et al., 2024). Bacteria employ spacer arrays with distinct gRNAs acquired from prior viral infections, enabling them to recognize and neutralize bacteriophages resembling previously encountered viral strains (Moreno-Mateos et al.,2015).

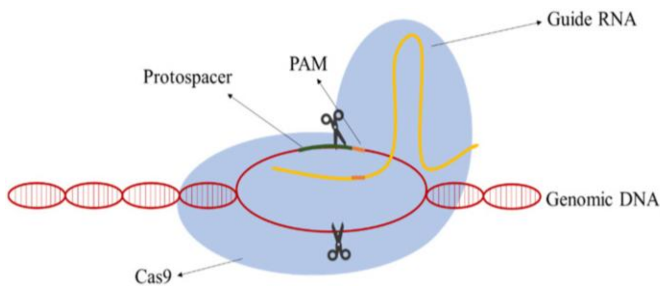

Figure 2. The mechanism of action of Cas9 is associated with short, regularly interspaced, and clustered palindromic repeats (CRISPR-Cas9). The guide RNA (gRNA) molecule directs the Cas9 protein to the target DNA and Cas9 cleaves the genomic DNA before the protospacer-adjacent motif (PAM) site (Rainha et al., 2020).

Three distinct CRISPR systems exist across a diverse range of bacterial hosts, each comprising a set of CRISPR-associated genes (Cas), non-coding RNAs, and a specific arrangement of repetitive elements (Samai et al., 2015). These repetitions are interspersed with short, variable sequences of 20 nitrogenous bases derived from exogenous DNA targets, known as protospacers, collectively forming the CRISPR RNA (crRNA) array (Koltun et al., 2023). Within the target DNA, each protospacer is linked to a protospacer adjacent motif (PAM), the specifics of which can vary depending on the particular CRISPR system (Lin et al., 2024) (Figure 2).

For optimal functionality, CRISPR necessitates specific ideal conditions for its application (Hu et al., 2014). The activity of Cas9 and the subsequent DNA repair processes may be influenced by the methylation state, altering the local chromatin structure. Moreover, the effectiveness of guide RNA (gRNA) can be subject to the cell cycle phase of the target cell. Another consideration is the fluctuating genomic stability observed across diverse cell types, which can contribute to gRNA efficacy (Koltun et al.,2023). The impact of DNA sequences flanking the PAM can affect both Cas9 binding and cleavage efficiency, as well as overall efficacy, given its propensity to slide towards PAMs, thus significantly influencing gRNA activities (Lin et al., 2024).

RNA-guided endonuclease CRISPR-Cas9

The first Cas protein used for genetic editing was Cas9, from *Streptococcus pyogenes*, *in vitro* and later in eukaryotic cells (Jinek et al., 2012). Cas9 is guided by two types of RNA, crRNA and tracrRNA (trans-activating RNA), to target double-stranded DNA. In cells, a single Cas9 enzyme, together with the sgRNA, forms a ribonucleoprotein (RNP) that scans the genome in search of a specific PAM sequence (Sternberg et al., 2014). The PAM sequence specific to SpCas9 is “NGG” and , less frequently, “NAG”, occurring every 4 to 8 nucleotides in the human genome and up to 41 bases in parasite genomes (Kalamakis; Platt, 2023). Without the presence of the PAM sequence, Cas9 does not confirm the target DNA, even if the sgRNA is fully matched (Caetano et al., 2018).

Cas9 enzymes from other species, known as orthologs, share this general mechanism, although they present differences in primary sequence, structure, and PAM requirements (Koonin; Makarova; Zhang, 2017). Upon encountering a PAM sequence, Cas9 promotes the separation of the DNA strands, allowing the crRNA to associate with the target sequence. If there is sufficient complementarity between the spacer sequence and the target strand, Cas9 undergoes a conformational change, positioning its two independent nuclease domains (RuvC and HNH) near the DNA strands, resulting in cuts in both (Kalamakis; Platt, 2023).

The HNH domain cuts the DNA strand complementary to the guide crRNA, while the RuvC domain cleaves the non-complementary, or coding, strand of the target DNA (see Figure 3). After creating DSBs, Cas9 retains the end containing the PAM until host enzymes remove the protein. Then, the cellular repair machinery either repairs the break, or if repair does not occur, the cell enters arrest or programmed cell death (Nussenzweig; Marraffini, 2020).

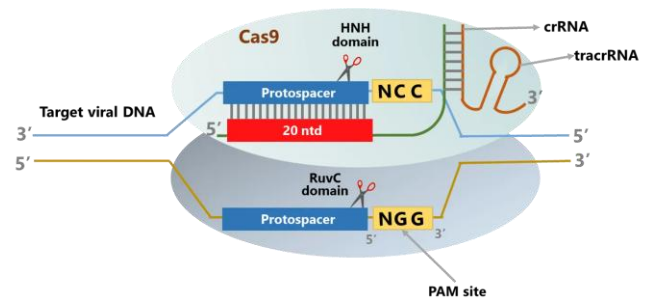

Figure 3. CRISPR-Cas9 system for genome editing, protospacer adjacent motif (PAM) for the Streptococcus pyogenes Cas9 nuclease (Bansal, 2022).

Additionally, other Cas9 proteins from different organisms (StCas9, NmCas9, FmCas9, and CjCas9) have been discovered, offering new research opportunities (Kalamakis; Platt, 2023). The main focus has been on finding smaller variants that are easier to manage or variants that support different PAM sequences, such as 5′NNG3′ for ScCas9 and 5′NNGG3′ for SauriCas9 (Anzalone; Koblan; Liu, 2020). Cas9 initiated a revolution in genetic editing, and despite some limitations, its benefits have driven profound advances in CRISPR biology, creating new technologies that are transforming the way biological systems are trained and manipulated (Kalamakis; Platt, 2023). The CRISPR/Cas9 technique can be applied in the laboratory to make precise modifications to the genes of various organisms, such as fruit flies, fish, mice, plants, and human cells (Caetano et al., 2018).

Agricultural Improvement

The CRISPR-Cas9 system has become a pivotal tool in deciphering transcriptional regulation, executing epigenetic modifications, and precisely visualizing specific genome loci under microscopic examination (Cheng et al., 2013). Continual modifications of this methodology aim to achieve diverse objectives, including gene replacement, chromosomal deletions, and the development of genetically modified plants without the reliance on markers (Hu et al., 2014, Samai et al., 2015).

Application in Soybeans (Glycine max (L) Merrill)

The scarcity of water during the pre-flowering phase can lead to a substantial, up to 40%, reduction in soybean productivity (Liu et al., 2023). Additionally, various biotic and abiotic stresses have been observed to negatively impact the growth and development of soybeans and other crops (Lu et al., 2023). The wild soybean variant (Glycine soja) stands out for its robust adaptability to saline and alkaline stresses, tolerating pH values up to 9.5. The strategic use of CRISPR in

introducing genes from wild varieties into cultivated species holds promise for significantly enhancing crop characteristics, particularly in mitigating water stress (Duanmu et al., 2015, Liu et al., 2023).

Liu et al., (2023) extensively discuss the pivotal roles of protein kinases in perceiving and transducing stress signals, highlighting the critical functions of SnRK1 kinases in plant tolerance to both biotic and abiotic stresses. By developing a snrk1.1-null lineage of Arabidopsis (herbaceous plant of the Brassicaceae family) through the CRISPR/Cas9 approach, the study reveals that the activity of the GsSnRK1.1 kinase (Glycine soja with snrk1.1 kinase) is crucial in enhancing water retention ability and survival rates under water stress, potentially through the timely regulation of stomatal closure. Active GsSnRK1.1 prevents water evaporation by mediating proper stomatal closure after water stress. Furthermore, the GsSnRK1.1 kinase is associated with stimulating root growth, and its phosphorylation activity plays a fundamental role in plant tolerance to water stress (Chen et al., 2021).

The study strongly recommends the validation of CRISPR-Cas systems through transient assays, particularly when the target species poses challenges for transformation (Koltun et al., 2023). The authors emphasize the validation of CRISPR-Cas systems and different gRNAs in BRS 537 soybean embryos, employing multiplexed gRNAs to induce larger DNA deletions with two gRNAs directing Cas9 to the same target gene. Notably, CRISPR systems offer a distinct advantage, as two gRNAs can be designed to induce partial gene deletion, enabling straightforward plant tracking through a simple PCR reaction (Liang et al., 2017). According to Koltun et al., (2023), confirmed the functionality of the proposed vector in soybeans, with the designed gRNAs applied for both single mutation and genetic deletion. This method effectively amplifies the editing signal of a transiently expressed CRISPR system, facilitating early and straightforward detection of genetic editing. Several strategies have been used to increase efficiency (Oliveira *et al.*, 2024).

Advancements in Potato Genome Editing Using CRISPR Technology

In the realm of genetic research aimed at optimizing genome editing efficiency, the CRISPR/Cas9 methodology has emerged as a pivotal tool (Samai et al., 2015). Specifically, improvements to the codon and promoter of the Cas9 gene expressed in various crops offer a strategic avenue for enhancing Cas9 expression levels, ultimately leading to improved editing efficacy (Ye et al., 2023). The utilization of genome editing provides rapid and efficient technical approaches to augment specific traits and accomplish precise molecular modifications (Lucioli et al., 2022).

In a groundbreaking study focusing on genome editing efficiency through CRISPR/Cas9 in potatoes, Ye et al., (2023) implemented carefully calibrated sodium chloride (NaCl) conditions and osmotic stress, resulting in a novel procedure. This approach, involving saline and osmotic stress, demonstrated a substantial increase in positive rates of genetic transformation and genome editing efficiency by CRISPR/Cas9 in potatoes, all without inducing undesirable off-target effects. Such innovation presents a promising and practical methodology for enhancing genome editing efficiency within CRISPR/Cas9 systems applied to potato cultivation.

Generating plants with a transgene-free genome is achievable through the CRISPR-Cas9 technique. Opting for a system that circumvents the integration of foreign DNA, as suggested by Kim et al*.,* (2018), is particularly relevant. CRISPR-Cas9 has been instrumental in enhancing potatoes, employing RNA-protein (RNP) delivery into protoplasts to minimize the presence of undesired insertions in the lineage. Andersson et al., (2018) employed RNP with mutations induced by synthetically produced RNA (CR-RNP). Under polyethylene glycol (PEG)-mediated transfection conditions, the mutagenesis rate reached an impressive 40%, with a frequency as high as 9%, and all lines were devoid of transgenes. This outcome aligns with observations in other crops, such as wheat (Liang et al., 2017) and maize (Svitashev et al., 2016), where frequencies ranged from 2-4% to 2–10%, respectively. Thus, the delivery of RNP through CRISPR-Cas9 technology holds considerable promise in generating commercial lines without unintended DNA incorporation.

The imperative to establish a transgene-free genome has spurred the development of innovative techniques. Veillet et al., (2019) employed Cytidine Base Editors (CBE), derived from CRISPR/Cas9, for the targeted conversion of cytidine bases to thymine. Stable genomic integration of CRISPR/Cas9 components, mediated by Agrobacterium, yielded efficient editing at target cytidine bases. The result was chlorsulfuron-resistant plants, with a remarkable base editing efficiency of up to 71% in tomatoes. In the first generation of tomatoes and potatoes, 12.9% and 10% of plants, respectively, were edited without transgenes. These findings underscore the potency of CRISPR technology in generating plants free from foreign DNA, marking a significant stride in the pursuit of genome editing precision.

Application in Wheat (Triticum aestivum L.)

Wheat is a vital crop, and the hexaploid nature of its genome makes it a model for studying and improving genome editing systems (Sánchez-león et al., 2018). Abiotic stress conditions pose significant challenges for wheat producers, resulting in substantial annual losses in yield in tons. Plants respond to these abiotic stress conditions through a comprehensive signaling mechanism involving the expression of various genes (Zhang et al., 2017). DREB2 (Dehydration-Responsive Element-Binding protein 2) and ERF3 (Ethylene-Responsive Factor 3) are key genes for abiotic stress tolerance, especially in drought situations (Kim et al., 2018, Zhang et al., 2017).

Kim et al., (2018) employed the CRISPR/Cas9 system for two abiotic stress-responsive transcription factor genes, TaERF3 and TaDREB2, in wheat protoplasts. Effective expression of guide RNA constructs (sgRNA-Cas9) was achieved for both TaDREB2 and TaERF3, with successful transient expression of sgRNA-Cas9 in wheat protoplasts. According to the study, to ensure the safety of this technique, off-target mutations were analyzed, revealing that genome editing outside the target sequence with a PAM sequence corresponding to TaDREB2 was significantly lower compared to the on-target sequence. The results support the specificity of genome editing with the CRISPR/Cas9 system in wheat, demonstrating efficiency in editing the genome for improved performance.

CRISPR is also used to produce non-genetically modified wheat with low gluten content, with the group of proteins α-

gliadins being responsible for the development of celiac disease (Ozuna et al., 2015). Sánchez-león et al., (2018) applied this technology to reduce the amount of α-gliadin in the grain (a component of the soluble fraction of gluten responsible for bread extensibility). Two guide RNAs (sgAlpha-1 and sgAlpha-2) were used to modify the genes. The T1 sgAlpha-1 lineage of bread wheat obtained the greatest reduction, with an 82% reduction in α-gliadins, 92% in γ-gliadins, and an overall reduction of 82% in gliadins. Therefore, gluten immunoreactivity can be reduced through the editing of α-gliadin genes with the 33-mer immunodominant epitope, a peptide predominant in celiac patients, as per the results obtained by Sánchez-león *et al.,* (2018). Demonstrating the effectiveness of CRISPR in simultaneously mutating the α-gliadin genes and reducing the immunoreactivity of edited wheat lines by 85%.

Originally derived from a bacterial resistance process, CRISPR can also be used to develop resistance to pests and diseases, including powdery mildew, which affects bread wheat production by reducing grain yield (Sánchez-martín et al., 2016). According to Zhang et al., (2017), in experiments with hexaploid wheat (Triticum aestivum) cloning TAEDR1 (a kinase protein that plays a negative role in powdery mildew resistance), they identified that the mutant could increase resistance to powdery mildew. Using CRISPR to generate edr1 plants by mutating three homologs of TaEDR1, the synchronous knockout of the three homologs imparts resistance to powdery mildew. Demonstrating that resistance is independent of cell death, and edr1 plants also showed resistance to bacterial and oomycete infections; analyses did not identify off-target mutations outside the Taedr1 target genome.

Application in Rice (Oryza sativa)

Highlighting its expansive utility, CRISPR can be harnessed to instigate metabolic alterations, yielding improvements not only in crops but also in human well-being. In a study by Abe et al., (2018) on rice (cv. Nipponbare), aiming to boost the concentration of oleic acid (known for its health benefits), the researchers employed the fatty acid desaturase 2 (FAD2) enzyme catalyzing oleic acid to linoleic acid. Through the application of the CRISPR/Cas9 system to knock out the FAD2-1 gene, they achieved a 10% reduction in linoleic acid content and a corresponding 10% increase in oleic acid in the heterozygous FAD2-1 rice.

In another study, Caddell et al., (2023) utilized genetic enhancement to craft three rice lineages with truncated light-harvesting antennas (TLA), each harboring a null mutation in the components of the rice CpSRP (chloroplast signal recognition particle) pathway: CpSRP43 (TLA3), CpSRP54a (TLA4), and CpSRP54b (TLA4L). Employing CRISPR/Cas9, they induced TLA3 and TLA4 mutants with reduced light-harvesting antennas and lower chlorophyll content. The observed increase in quantum yield and solar energy conversion capacity suggests that reducing chlorophyll is a viable strategy to enhance photosynthetic efficiency, promoting effective light distribution to lower leaves and minimizing non-photochemical waste.

Application in Tomato (Solanum lycopersicum)

Tomato cultivation is highly influenced by climate conditions, with cold stress being a significant abiotic factor restricting its development and productivity (Wang et al., 2017). In an experiment conducted by Li et al., (2018), CRISPR/Cas9 was employed to generate slcbf1 mutants (CBF family gene related to water stress tolerance) to assess their relationship with cold tolerance in tomatoes. The study reported high CRISPR efficiency with no off-target mutations. The slcbf1 mutant plants exhibited severe chilling injuries and reduced expression of CBF-mediated genes. Understanding the action of the slcbf1 gene is crucial for directing new studies aimed at enhancing cold tolerance mediated by CBF. Therefore, fundamental studies on gene action are essential for identifying and reproducing improvements in crops, with CRISPR playing a pivotal role in this process.

Application in Cassava (Manihot esculenta Crantz)

Cassava, vital for food security due to its high drought tolerance, faces challenges in post-harvest deterioration through root injuries (Zeng et al., 2020b). Scopoletin, synthesized by the enzyme feruloyl CoA 6'-hydroxylase, is a key metabolite during this deterioration phase. In studies, Mukami *et al.,* (2024) utilized the CRISPR/Cas9 system to target the MeF6′H1, MeF6′H2, and MeF6′H3 genes encoding this enzyme, aiming to reduce scopoletin concentration. Mutated lineages demonstrated reduced scopoletin levels and increased post-harvest shelf life. The double knockout of genes extended the harvest's validity period between 21 to 24 days. Thus, the CRISPR methodology plays a crucial role in genetic improvement processes, contributing to the enhancement of crops.

Application in Sugarcane (Saccharum officinarum L.)

Sugarcane, with significant production potential under tropical conditions and cultivated in approximately 100 countries, holds a vital role in expanding the clean and renewable energy matrix (Oliveira et al., 2022). Pokkah Boeng disease, caused by the Fusarium species, adversely affects sugarcane's young leaves, impacting its development and, in severe cases, leading to cane tip necrosis and plant death (Lin et al., 2014b). To develop a system for detecting genes related to this disease in a single reaction, Zhu et al., (2024) combined CRISPR/Cas12 and Loop-Mediated Isothermal Amplification (LAMP) technologies, coining it Cas-mCfLAMP. Results indicated that the Cas-mCfLAMP technique not only facilitated rapid and straightforward diagnosis in sugarcane under laboratory conditions but also in the field, as it eliminates the need for separate pathogen detection

Conclusions

CRISPR-Cas9 technology stands out as a promising tool for genetic editing across various domains, encompassing the functional characterization of genes to enhance agricultural crops. It is a cost-effective and highly efficient methodology. Its application in understanding transcriptional regulation, epigenetic modifications, and the creation of genetically modified plants highlights its positive impact in various biotechnological research fields. Beyond its comprehensive genetic approach, CRISPR is valuable for its ability to avoid off-target mutations, adding substantial value to the technique. Its use is indispensable in the pursuit of significant advances in genetic manipulation, fostering innovation, and contributing to scientific and agricultural progress.

This technique has facilitated remarkable strides in the development of crop varieties, whether through gene mutation

for increased resistance or the refinement of crops to meet specific needs. It plays a pivotal role in boosting agricultural productivity, enabling the creation of crops adapted to adverse environments, and significantly enhancing food security. CRISPR is employed collaboratively in both the health and agricultural sectors, facilitating an essential synergy in the pursuit of well-being. CRISPR/Cas9 thus represents a fundamental tool in genetic engineering, propelling significant innovations to address global agricultural challenges. The technique still holds ample potential for further evolution, necessitating more extensive studies.

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