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CRISPR-Cas revolution in Agriculture: From precision genome editing to sustainable crop improvement

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ABSTRACT

The CRISPR-Cas system has emerged as a transformative tool in agricultural biotechnology, revolutionizing the landscape of crop improvement. This review paper explores the multifaceted applications of CRISPR technology in agriculture, from its fundamental principles to its practical implementations for precision genome editing. We delve into the various strategies employed to enhance crop growth and yield traits, including disease resistance, abiotic stress tolerance, and nutritional content, thereby contributing to the development of sustainable agriculture practices. Furthermore, we discuss the regulatory frameworks and ethical considerations surrounding the deployment of CRISPR-edited crops, highlighting the challenges and opportunities for its widespread adoption. Through a comprehensive analysis of recent advancements and future prospects, this review aims to provide insights into the role of CRISPR-Cas in shaping the future of agriculture and global food security.

KEY WORDS: CRISPR; Cas9; Agriculture; Gene editing

1. Introduction

The application of CRISPR-Cas9 technology in agriculture has catalyzed a paradigm shift in crop improvement strategies, offering unparalleled precision and efficiency in genome editing. CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) and its associated protein Cas9 constitute a groundbreaking molecular toolkit that enables targeted modifications of DNA sequences within plant genomes (Jinek *et al.*, 2012). This revolutionary genome editing platform has not only expedited the pace of genetic manipulation in crops but also unlocked novel avenues for sustainable agriculture and global food security (Hsu *et al.*, 2014).

The versatility and simplicity of the CRISPR-Cas9 system have propelled research efforts worldwide, driving significant advancements in crop biotechnology. By precisely targeting genes linked to agriculturally significant traits, such as yield, stress tolerance, and nutritional composition, researchers have achieved remarkable success in developing crops with enhanced productivity and resilience (Puchta and Fauser, 2014). For instance, CRISPR-mediated editing has been instrumental in conferring resistance to devastating pathogens like powdery mildew in wheat (Wang *et al.*, 2014), bacterial blight in rice (Li *et al.*, 2012), and citrus canker in citrus plants (Jia and Wang, 2014).

Moreover, CRISPR technology holds promise for

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promoting sustainable agricultural practices by mitigating the environmental impacts associated with conventional farming methods. Through targeted genome editing, researchers can engineer crops that require fewer chemical inputs, such as pesticides and fertilizers, thereby reducing environmental pollution and preserving soil health (Schindele *et al*., 2018). Additionally, CRISPR-edited crops with improved nutritional profiles offer a viable solution to malnutrition and food insecurity, particularly in regions where staple crops lack essential vitamins and minerals (Wurtzel *et al*., 2019).

Despite the transformative potential of CRISPR-Cas9 in agriculture, several challenges persist, ranging from regulatory complexities to ethical considerations and public acceptance (Waltz, 2016). The regulatory landscape governing the cultivation and commercialization of genetically modified organisms (GMOs) varies across jurisdictions, presenting obstacles to the global adoption of CRISPR-edited crops (Chawla *et al*., 2017). Moreover, concerns regarding unintended off-target effects and the potential for gene flow to wild relatives underscore the need for rigorous risk assessment and environmental monitoring (Bortesi and Fischer, 2015).

In this comprehensive review, we aim to provide an extensive exploration of the CRISPR-Cas revolution in agriculture, spanning its diverse applications, underlying mechanisms, regulatory frameworks, and ethical implications. By synthesizing recent research findings and emerging trends, we seek to elucidate the transformative potential of CRISPR technology in driving sustainable crop improvement and addressing the multifaceted challenges confronting modern agriculture.

2. CRISPR-Cas: components and mechanism

2.1 Components of CRISPR-Cas system

At its core, the CRISPR-Cas system consists of two primary components: the Cas protein and the guide RNA (gRNA). The Cas protein, typically Cas9, serves as the molecular scissors responsible for cleaving DNA at specific target sequences. The gRNA, composed of a CRISPR RNA (crRNA) and a trans-activating CRISPR RNA (tracrRNA) fused together, guides the Cas protein to the target DNA sequence through complementary base pairing (Jinek *et al*., 2012).

2.2 Mechanism of CRISPR-Cas action

The mechanism of CRISPR-Cas-mediated genome editing involves several key steps. Initially, the gRNA forms a complex with the Cas protein, leading to the formation of the Cas-gRNA ribonucleoprotein (RNP) complex. This complex scans the genomic DNA for sequences complementary to the gRNA, facilitating target recognition (Doudna and Charpentier, 2014).

Upon binding to the target DNA sequence, the Cas protein undergoes a conformational change, resulting in the activation of its endonuclease activity. The endonuclease domains of the Cas protein then catalyze the cleavage of the DNA, generating double-strand breaks (DSBs) at the target site (Gasiunas *et al*., 2012).

Following DNA cleavage, the cell's DNA repair machinery comes into play to resolve the DSBs. Two primary pathways involved in DNA repair are non-homologous end joining (NHEJ) and homology-directed repair (HDR). NHEJ often leads to small insertions or deletions (indels) at the

site of the DSB, resulting in gene knockout or disruption. In contrast, HDR utilizes a template DNA molecule to precisely repair the DSB, enabling gene editing and insertion of desired sequences (Doudna and Charpentier, 2014).

3. CRISPR-Cas9: evolution to precision genome editing

The journey of the CRISPR-Cas system began with the elucidation of clustered regularly interspaced short palindromic repeats (CRISPR) in the genomes of bacteria and archaea. Initial studies in the late 1980s and 1990s identified these repetitive DNA sequences, which sparked curiosity about their function. However, it was not until the early 2000s that researchers began to unravel the significance of CRISPR in bacterial immunity against viral infections.

The versatility of CRISPR-Cas9 lies in its ability to target virtually any genomic locus by simply modifying the sequence of the gRNA. This programmable nature, coupled with its high efficiency and specificity, has revolutionized genome editing and facilitated the study of gene function and regulation in various organisms.

4. Importance of CRISPR-Cas in Agriculture

The escalating impacts of climate change have intensified the specter of food scarcity, necessitating innovative approaches to bolster agricultural productivity. Climatic shifts, characterized by erratic weather patterns, extreme temperatures, and unpredictable precipitation, have destabilized traditional farming practices, leading to reduced crop yields and compromised food security. In response to these challenges,

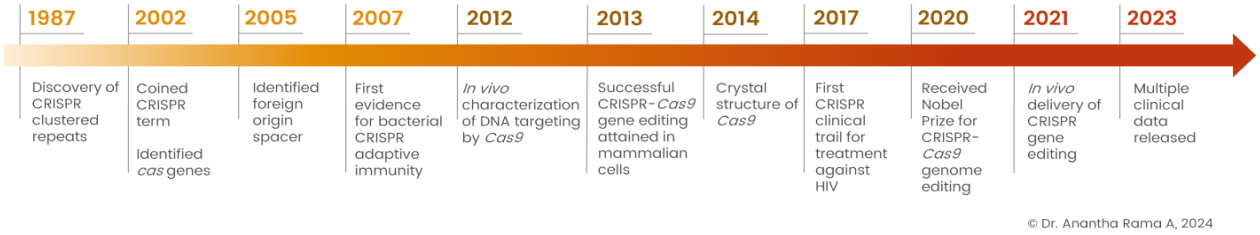

Fig. 1: Evolution of the CRISPR-Cas System: Key Milestones

The breakthrough came in 2012 when Doudna and Charpentier demonstrated the programmable nature of CRISPR-Cas9 for targeted genome editing in bacteria. Their landmark paper published in Science described how the Cas9 protein, guided by a short RNA molecule, could precisely cleave specific DNA sequences. This pivotal discovery laid the foundation for a myriad of applications in genetic engineering, ranging from gene knockout and knock-in to gene regulation and functional genomics.

targeted genome editing, facilitated by advanced molecular tools such as CRISPR-Cas systems, has emerged as a promising approach to boost agricultural productivity. In rice and wheat, targeted genome editing has been instrumental in increasing grain size, weight, and number, as well as enhancing protein content, tiller spread, and tiller number. These improvements have been reported in various studies (Wang *et al*., 2020; Oliva *et al*., 2019; Zhang *et al*., 2019).

Moreover, targeted genome editing has led to significant enhancements in the quality of crops such as rice and corn. Modified crops utilizing the CRISPR–Cas system have been tailored to reduce the levels of toxic steroidal glycoalkaloids, thereby enhancing the color and extending the shelf-life of fruits and vegetables, rendering them more commercially appealing. Additionally, these modifications have resulted in an increase in desirable traits such as amylose and starch content, as well as good fats like oleic acid levels. Furthermore, improvements in fragrance, gluten protein reduction, and decreased unsaturated fatty acids content have been achieved (Li *et al*., 2012; Cermak *et al*., 2015; Clasen *et al*., 2016; Jia *et al*., 2017). These advancements highlight the transformative potential of CRISPR-based targeted genome editing in agriculture, offering precise and tailored solutions to address the complex challenges posed by climate change and food insecurity.

4.1 Enhancing crop yield and quality

CRISPR-Cas9 genome editing, targeting the OsNAS2 promoter, specifically deleting the cis-regulatory element ARR1AT at position -933, significantly increased Zn concentration per plant in rice and also led to an augmented spikelet number per main panicle, resulting in increased grain yield per plant (Ludwig *et al.*, 2024). In an another study conducted by Usman *et al*. (2020) reported that precise editing of the OsPYL9 gene by RNA-guided Cas9 nuclease increased the grain yield in rice by regulating circadian rhythm. CRISPR/Cas9-mediated multiplex genome editing targeted three key genes - GW2, GW5, and TGW6, known as negative regulators of grain weight and the outcomes demonstrated a notable increase in grain size and thousand grain weight (Xu *et al*., 2016).

Bioactive compounds, characterized as additional nutritional constituents found in small quantities in foods, often contribute to the prevention of cardiovascular disease and cancer. Anthocyanin, malate, γ-aminobutyric acid (GABA), and lycopene are among these bioactive compounds. Utilizing CRISPR-Cas9 technology, researchers have enhanced the levels of anthocyanin, GABA, and lycopene in tomato fruits by modulating the expression of key genes in their metabolic pathways (Cermak *et al*., 2015; Nonaka *et al.*, 2017).

The function of TM6 in strawberry was elucidated using the CRISPR-Cas9 system applied to an octoploid species. Phenotypic analysis of tm6 mutants unveiled pronounced defects in anthers, underscoring TM6's crucial role in flower development (Martín-Pizarro *et al.*, 2019). Furthermore, CRISPR-Cas9 was employed to explore the biological role of YUCCA 10 (YUC10) in auxin synthesis during strawberry fruit development. Knocking out YUC10 resulted in a significant reduction in free auxin in yuc10 mutants (Feng *et al*., 2019).

4.2 Disease resistance

CRISPR–Cas13a presents an efficient tool for targeting RNA viruses, predominantly plant viruses. Aman *et al.* 2018 utilized LshCas13a to target Turnip mosaic virus (TuMV) which cause Turnip mosaic disease in *Nicotiana benthamiana*, achieving significant reductions in viral gene expression. The predominant approach for pathogen control via the CRISPR/Cas9 system involves disrupting the host's susceptibility gene (S gene), thus impeding plant-pathogen

interactions and preventing pathogen establishment (Zaidi *et al*., 2018). This disruption can be achieved by targeting either the promoter sequence of the S gene or interrupting the effector-binding site. Ali and his coworkers effectively demonstrated virus targeting by inducing indels in the genome of tomato yellow leaf curl virus, thus imparting viral resistance. This resistance was achieved through CRISPR/Cas9 binding to the viral genome, subsequently obstructing the viral genome's access to replication units, or by generating blunt-end cuts or indel mutations on the viral genome. Thomazella *et al*. (2016) utilized the CRISPR-Cas9 system to deactivate the DMR6 ortholog in tomatoes. The resulting dmr6 mutants exhibited disease resistance against a range of pathogens, such as *Pseudomonas syringae*, *Phytophthora capsica*, and *Xanthomonas* spp., with minimal adverse effects. *Pseudomonas syringae* induces bacterial speck disease in tomato plants, which significantly impacts their productivity and market value. Given the role of Jasmonatezim domain protein 2 (JAZ2) in defense against *P. syringae* in *A. thaliana*, scientists employed CRISPR-Cas9 to produce dominant JAZ2 repressors in tomatoes with the C-terminal jasmonate associated (Jas) domain removed (JAZ2Δjas). These JAZ2Δjas repressors confer resistance to *P. syringae*. Nekrasov *et al*. (2017) employed CRISPR-Cas9 technology to create a tomato loss-of-function mlo1 mutant. This mutant exhibited complete resistance to the powdery mildew fungus *Oidium neolycopersici*.

4.3 Herbicide resistance

The application of the CRISPR-based gene editing technique has led to the successful development of crop varieties resistant to herbicides that target the ALS enzyme. This technique has been implemented across various crops, such as rice (Zhang *et al*., 2021), maize (Li *et al*., 2020), wheat (Zhang *et al*., 2019), watermelon (Tian *et al*., 2018), oilseed rape (Wu *et al*., 2020), tobacco (Kang *et al*., 2019), tomato and potato (Veillet *et al*., 2019). Additionally, wheat has shown tolerance to herbicides inhibiting ACCase through cytidine-deaminase-mediated base editor (CBE). To enhance the efficiency of CRISPR/Cas technology, the target-activation induced cytidine deaminase (Target-AID) system has been introduced, facilitating the simultaneous improvement of multiple traits in crops.

In the development of herbicide-resistant crop varieties, only resistance to ALS-inhibiting herbicides, ACCase-inhibiting herbicides, and glyphosate has seen significant success. However, research on the widespread adoption and effective management of weeds with herbicides that target 4-hydroxyphenyl pyruvate dioxygenase and protoporhyrinogen oxidase is lacking.

4.4 Plant stress resistance

Stress poses a formidable challenge to agricultural productivity, with abiotic and biotic stressors exerting detrimental effects on crop yield. Abiotic stressors, encompassing factors such as drought, floods, temperature extremes, salinity, heavy metals, and radiation, disrupt plant growth and development. Conversely, biotic stress arises from attacks by various pathogens including viruses, bacteria, fungi, and herbivores, further compromising crop health and productivity. To mitigate these challenges, crops such as rice, tomato, cucumber, and grapefruits have been genetically modified through induced mutations to enhance resistance to both abiotic (Klap *et al*.,

2017) and biotic stresses (Lu *et al*., 2018). While earlier attempts at site-specific genomic mutation relied on DNA-binding endonucleases such as zinc finger nucleases (ZFN) and transcription activator-like effector nucleases (TALEN), these approaches have inherent limitations (Christian *et al*., 2010). The advent of the CRISPR–Cas system marked a significant breakthrough, enabling precise genome editing in a wide range of crops including rice, wheat, *Nicotiana benthamiana*, and *Arabidopsis* (Chen *et al*., 2019). In their study, Li *et al.* (2018) discovered that C-repeat binding factor 1 (CBF1) plays a crucial role in safeguarding plants against cold injury. The cbf1 mutant, created using CRISPR-Cas9, displayed exacerbated chilling-injury symptoms with

Table 1: CRISPR-Based crop improvement studies in important agriculture and horticulture crops.

Crops	Targeted gene	Result	References
Agricultural crops			
Rice	*OsSEC3A, OsSWEET13, OsERF922*	Resistant to blast and bacterial blight	Ma *et al*., 2018
Rice	*ALS*	Herbicide resistance	Chen *et al*., 2019
Rice	*UVb1-1*	Resistant to false smut	Mishra *et al*., 2018
Rice	*OsGS3*	Increase in grain size	Miao *et al*., 2013
Wheat	*EDR1*	Resistant to powdery mildew	Zhang *et al*., 2017
Barley	*ENGase, HvPM19, BolC.GA4.a*	Increase in number of grains	Kapusi *et al*., 2017
Maize	*ARGOS8*	Drought resistance	Svitashev *et al*., 2016
Horticultural crops			
Tomato	*SlMLO1*	Resistant to powdery mildew	Nekrasov *et al*., 2017
Potato	*ALS*	Herbicide resistance	Choudhury *et al*., 2016
Cucumber	*eIF4E*	Broad virus resistant	Sauer *et al*., 2016
Apple	*DIPM1, DIPM2, DIPM4*	Resistant to fire blight disease	Malnoy *et al*., 2016
Kiwifruit	*CEN4, CEN*	Rapid flower and fruit development	Varkonyi *et al*., 2018
Grape	*VvMLO3*	Resistant to powdery mildew	Wan *et al*., 2020
Citrus	*CsLOB1*	Resistant to citrus canker	Jia *et al*., 2017
Cocoa	*TcNPR3*	Resistant to *Phytophthora tropicalis*	Fister *et al*., 2018
Watermelon	*ALS*	Herbicide resistance	Tian *et al*., 2018
Papaya	*alEPIC8*	Resistance to *Phytophthora palmivora*	Gumtow *et al*., 2018
Cassava	*EPSPS*	Herbicide resistance	Hummel *et al*., 2018
Soybean	*GmSPL9a, b, c*	Increase in yield	Bao *et al*., 2019
Mushroom	*PPO*	Browning resistant	Waltz, 2016

increased electrolyte leakage compared to wild-type (WT) plants. Additionally, MAPK3, known for its involvement in resisting gray mold disease (Zhang *et al*., 2018), also contributes to tomato drought response by shielding cell membranes from oxidative damage.

Customized sgRNA-Cas9 systems have emerged as a widely employed tool for genome modification in crops like rice and wheat, showcasing the ease and efficiency of genome editing (Shan *et al*., 2013). Notably, Cas12a, formerly known as Cpf1, presents advantages over Cas9 in plant genome editing due to its requirement of shorter guiding nucleotides, ability to create larger deletions at target sites, and facilitation of NHEJ-mediated donor DNA insertion (Kim *et al*., 2017).

In *Arabidopsis*, Feng and coworkers successfully demonstrated the mutation and heritability of five endogenous target genes – *brassinosteroid insensitive 1 (bri1), jasmonate-zim-domain protein 1 (jaz1), gibberellic acid insensitive (gai), magnesium chelatase subunit i (chli),* and *transparent testa 4 (tt4)*, in addition to the *apetala1* (*ap* 1) gene, using CRISPR–Cas tools (Feng *et al*., 2014). Furthermore, CRISPR–Cas technology can be harnessed for the regulation of genes responsible for epigenetic modification, methylation, and/or demethylation, enabling simultaneous induction and repression of gene expression (Puchta, 2016).

Hybrid breeding, alongside precision plant breeding facilitated by CRISPR–Cas, holds promise for increasing crop productivity (Chen *et al*., 2019). CRISPR–Cas has been instrumental in producing thermosensitive male-sterile lines in rice (Zhou *et al*., 2016) and maize (Svitashev *et al*., 2016), facilitating the production of high-quality hybrid varieties. Additionally, knockout mediated by CRISPR–Cas has enabled the development of herbicide-resistant crops in rice (Shimatani *et al*., 2017), *Arabidopsis* (Chen *et al*., 2017), and watermelon (Tian *et al*., 2018), further expanding the scope of genome editing applications in plants.

5. CRISPR crop regulations and ethics

The regulatory framework surrounding CRISPR-modified crops varies significantly among countries and regions. In some jurisdictions, CRISPR-edited crops that do not involve the insertion of foreign DNA are subject to less stringent regulations compared to traditional genetically modified organisms (GMOs). For example, the European Union (EU) has classified some CRISPR-edited crops as non-GMOs, thereby exempting them from rigorous regulatory requirements (Eckerstorfer *et al*., 2019).

In contrast, other countries, such as the United States, have adopted a case-by-case approach to regulate CRISPR-modified crops, evaluating them based on their characteristics and potential risks to human health and the environment. The U.S. Department of Agriculture (USDA), the Environmental Protection Agency (EPA), and the Food and Drug Administration (FDA) play key roles in assessing the safety and environmental impact of CRISPR-modified crops (Waltz, 2018).

5.1 Ethical Implications

CRISPR-mediated genome editing in agriculture raises various ethical considerations that must be carefully addressed. One of the primary concerns revolves around unintended consequences and potential ecological impacts of genetically

modified crops. Altering genes in crops could inadvertently affect ecosystems, biodiversity, and non-target organisms, leading to unforeseen environmental consequences (Lassoued *et al.*, 2019).

Additionally, ethical considerations extend to issues of social justice and equity in access to CRISPR technology and its benefits. There is a risk that CRISPR-based agricultural innovations could exacerbate existing inequalities, favoring large agro-industrial companies and marginalizing small-scale farmers and resource-constrained regions. Ensuring equitable access to CRISPR technology and its benefits is essential for promoting social justice and addressing global food security challenges (Levidow and Carr, 2020).

Furthermore, questions surrounding informed consent, transparency, and public engagement in decision-making processes related to CRISPR-modified crops are paramount. Stakeholder involvement, including farmers, consumers, policymakers, and civil society organizations, is crucial for fostering transparency, accountability, and democratic governance in agricultural biotechnology.

6. Conclusion

The advent of CRISPR-Cas technology heralds a new era in agriculture, offering unparalleled precision, efficiency, and adaptability in crop enhancement. With its transformative potential, CRISPR-Cas has emerged as a powerful tool for addressing the multifaceted challenges confronting contemporary agriculture. By leveraging the capabilities of CRISPR-Cas, researchers are poised to revolutionize crop breeding practices, enabling the development of resilient, high-yielding, and nutritionally enriched cultivars.

CRISPR-Cas-mediated genome editing facilitates the targeted modification of specific genes, thereby accelerating the breeding process and circumventing the limitations of traditional breeding methods (Zhang *et al.*, 2019). This precision breeding approach holds tremendous promise for enhancing crop traits such as disease resistance, abiotic stress tolerance, and nutritional quality, thereby bolstering agricultural productivity and resilience in the face of climate change and environmental pressures (Wang *et al.*, 2020). Furthermore, the versatility of CRISPR-Cas extends beyond genetic modification to encompass epigenome editing and gene regulation, offering novel avenues for crop improvement (Shimatani *et al.*, 2017). By precisely modulating gene expression patterns and regulatory networks, CRISPR-Cas9 enables fine-tuning of agronomically important traits, such as flowering time, yield components, and nutrient utilization efficiency.

Moreover, CRISPR-Cas technology holds immense potential for promoting sustainable agriculture and addressing global food security challenges (Nalley *et al.*, 2019). By enhancing crop productivity, reducing input requirements, and minimizing environmental impacts, CRISPR-edited crops offer a pathway towards achieving food security goals while mitigating the ecological footprint of agricultural production systems.

In conclusion, CRISPR-Cas technology represents a paradigm shift in agriculture, offering unprecedented opportunities for crop improvement and sustainable development. By

harnessing the power of CRISPR-Cas, researchers can accelerate the pace of genetic improvement in crops, cultivate resilience to environmental stresses, and contribute to the realization of a food-secure future for generations to come.

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