CRISPR-Cas-Based Genome Editing for Crop Improvement: Progress, Challenges and Future Prospects

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Abstract: The discovery of the CRISPR-Cas genome editing technology has opened up new opportunities for crop improvement through precise genetic modifications. This new technology has shown great promise in improving crop yields, quality, and resilience to biotic and abiotic stresses. This review presents the recent advances in CRISPR-Cas technology, including new tools and techniques for precise genome editing, as well as the challenges associated with off-target effects and unintended consequences. It explores the applications of CRISPR-Cas-based genome editing in different crops, including maize, rice, wheat, and tomato, highlighting the progress achieved in improving important traits such as disease resistance, drought tolerance, and nutrient content. The regulatory concerns around CRISPR-Cas-based genome editing, as well as the ethical considerations associated with this technology are also adressed. Finally, insights into the potential impact of CRISPR-Cas-based genome editing on crop breeding and food security, and the challenges that need to be addressed to fully realize its potential are provided. This review thus highlights the potential of CRISPR-Cas-based genome editing in crop improvement and emphasizes the importance of continued research in this area for sustainable agricultural production.

Keywords: CRISPR-Cas, Genome editing, Crop improvement, Progress, Challenges, Future prospects.

1. INTRODUCTION

Genome editing using CRISPR-Cas technology has emerged as a powerful tool for crop improvement. This technology allows for precise modifications of the plant genome, enabling the introduction of beneficial traits such as increased yield, disease resistance, and stress tolerance (Biswas *et al*., 2021 ; Kumar *et al*., 2021 ; Wang *et al*., 2022 ; Pan *et al*., 2023). CRISPR-Cas-based genome editing has the potential to revolutionize agriculture and contribute to global food security. In recent years, there have been significant advancements in the application of CRISPR-Cas technology in crop improvement. Several crops, such as rice, wheat, soybean, and maize, have been genetically modified using CRISPR-Cas technology, with promising results in terms of improved traits and yields (Li *et al*., 2016; Qaim, 2020 ; Ahmad *et al*., 2021; Abdelrahman *et al*., 2021 ; Ahmad, 2023). However, there are also challenges and ethical considerations associated with the use of CRISPR-Cas technology in crop improvement. The regulatory landscape for genome editing in crops is complex and varies by country, and there are concerns about the potential unintended effects of genetic modifications and their impact on the environment and food safety (Ahmad *et al*., 2021 ; Eckerstorfer *et al*., 2019 ; Qaim, 2020).

In this article, we review the progress, challenges, and future prospects of CRISPR-Cas-based genome editing in crops. We discuss the advantages of this technology over traditional breeding methods, highlight successful applications of CRISPR-Cas technology in crop improvement, and examine the challenges associated with the use of this technology in crops. It also explores the potential impact of CRISPR-Cas technology on agriculture and food security in the future and discuss the need for responsible use of genome editing technology to ensure public acceptance and sustainability in agriculture.

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2. METHODOLOGY

The review strategy employed in this work consisted of a comprehensive literature search of relevant studies related to CRISPR-Cas technology and its application in genome editing for crop improvement. The search was done using virtual databases such as Google Scholar, PubMed, Scopus, and Web of Science etc. The following are some of the search terms and keywords used in several mixtures : "CRISPR-Cas", "Genome editing", "Crop improvement", "Genetic progress", "Technological Challenges", and "Future prospects" as well as associated phrases. The search was limited to articles published in English and French, focused on studies carried out in the area of biotechnology and the application of CRISPR-Cas technology. In addition to the virtual database search, physical libraries were consulted for references in the domain. This backward citation tracking methodology helped to ensure a holoistic review of the relevant literature.

3. CRISPR-CAS TECHNOLOGY

CRISPR-Cas technology is a powerful gene-editing tool that uses RNA molecules to target specific DNA sequences and the Cas enzyme to cut the DNA at those sites (Doudna & Charpentier, 2014). This allows scientists to make precise changes to the genome, such as adding, removing, or replacing specific DNA sequences. Engineered CRISPR systems contain two components: a guide RNA (gRNA or sgRNA) and a CRISPR-associated endonuclease (Cas protein). The gRNA is a short synthetic RNA composed of a scaffold sequence necessary for Cas-binding and a user-defined ~20 nucleotide spacer that defines the genomic target to be modified. Thus, one can change the genomic target of the Cas protein by simply changing the target sequence present in the gRNA (*e.g*. Figure **1**). One of the key advantages of CRISPR-Cas technology is its simplicity and versatility, which make it relatively easy to use and applicable to a wide range of organisms and cell types (Hsu *et al*., 2014 ; Anonymous, 2023).

In contrast, previous gene-editing tools like zinc finger nucleases and TALENs were more difficult to design and optimize. While CRISPR-Cas technology has shown great promise for a variety of applications, there are also concerns about the potential risks and ethical implications of its use (Liu *et al*., 2021). For example, there is a risk of off-target effects where the Cas enzyme cuts DNA at unintended sites, potentially causing unintended mutations and harm. To address these concerns, the scientific community has been developing new tools and methods to improve the precision and specificity of CRISPR-Cas editing (Xu & Li., 2020 ; Wienert & Cromer, 2022). For example, base editing can be used to make more precise changes to the DNA sequence. Overall, responsible use and regulation of CRISPR-Cas technology is crucial to avoid unethical and potentially harmful applications (Barrangou & Doudna, 2016). However, with continued development and refinement, the technology has enormous potential for both basic research and applied biotechnology.

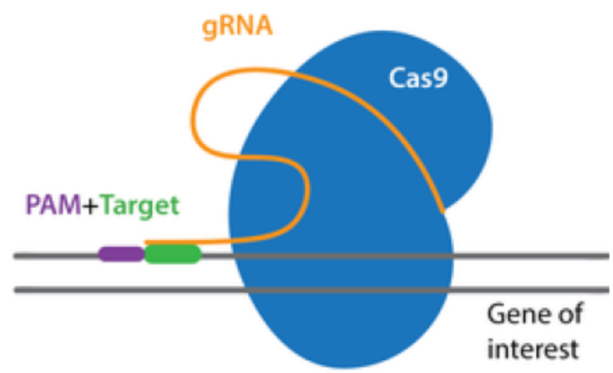

Figure 1: Engineered CRISPR systems with a guide RNA (gRNA or sgRNA) and a CRISPR-associated endonuclease (Cas protein) (Anonymous, 2023).

4. PROGRESS IN CRISPR-CAS-BASED GENOME EDITING IN CROPS

CRISPR-Cas technology has shown great potential for crop improvement, and several crops have been genetically modified using this technology with promising results. For example, researchers have used CRISPR-Cas to improve yield, disease resistance, and stress tolerance in crops such as rice, wheat, soybean, and maize (Wang *et al*., 2022 ; Li *et al*., 2016; Abdelrahman *et al*., 2021). In rice, CRISPR-Cas technology has been used to introduce mutations in genes resulting in plants with increased resistance to bacterial blight (Li *et al*., 2016). In wheat, CRISPR-Cas was used to introduce mutations resulting in plants with larger grains and increased yield (Wang *et al*., 2022). In soybean, researchers have used CRISPR-Cas to introduce mutations resulting in plants with increased salt tolerance (Wang *et al*., 2022). Moreover, CRISPR-Cas has been used to introduce mutations in plants, resulting in increased drought tolerance (Abdelrahman *et al*., 2021).

In addition, CRISPR-Cas technology has also been used to introduce more complex genetic modifications, such as gene replacements, insertions, and deletions, in crops. For example, CRISPR-Cas has been used to replace a gene in rice with a herbicide-resistant gene, resulting in plants that are resistant to the herbicide but have no other observable phenotypic changes (Li *et al*. 2016 ; Zaidi *et al*., 2020 ; Liu *et al*., 2021). Similarly, the CRISPR-Cas technology has been used efficiently in rice genome diting (Li *et al*., 2016 ; Zafar *et al*., 2020). These examples demonstrate the potential of CRISPR-Cas technology in crop improvement and suggest that this technology can be used to develop crops with desirable traits and increased productivity. Table **1** presents some examples of plants that have been modified using CRISPR-Cas technology.

These examples in Table **1** further demonstrate the potential and versatility of CRISPR-Cas technology for modifying a wide range of plant species and improving various plant traits, such as disease resistance, nutritional quality, plant architecture, yield, shelf life, and seed characteristics. Despite the regulatory and ethical concerns, these findings suggest that the technology could have significant implications for improving crop productivity and food security and that it may play an important role in advancing agricultural productivity and sustainability.

5. CHALLENGES IN CRISPR-CAS-BASED GENOME EDITING IN CROPS

Despite the potential of CRISPR-Cas technology, there are several challenges and ethical concerns associated with the use of this technology in crops. One major challenge is the regulatory landscape for genome editing in crops, which is complex and varies by country. In some countries, such as the United States, genome-edited crops may be regulated differently from conventionally bred crops, depending on the extent of genetic modification (Ahmad *et al*., 2021 ; Eckerstorfer *et al*., 2019 ; Qaim, 2020). This can

Table 1: Some Studies that have used CRISPR-Cas Technology in Crop Improvement

Plant	Objective / Results	Authors
Soybean	Editing of the fatty acid desaturase 2 gene in soybean.	Haun *et al*. (2014)
Brassica napus.	Enhancement of low-erucic acid in *Brassica.*	Liu *et al*. (2022)
Rice	Editing the Os BADH 2 gene in rice created fragrance. TALEN-based gene editing produces disease-resistant rice.	Shan *et al*. (2015) Li *et al*. (2012)
Tomato	Mutagenesis of the RIN locus that regulates fruit ripening in tomato.	Ito *et al*. (2015)
Wheat	Editing homoeoalleles of the susceptibility gene in wheat, conferring heritable resistance to powdery mildew.	Wang *et al*. (2014)
Potato	Modification of the potato genome to reduce the formation of acrylamide, a potential carcinogen.	Clasen *et al*. (2016)
Grape	Gene editing to enhance resistance to powdery mildew.	Nakajima *et al*. (2017) Wang *et al*. (2018)
Oilseed rape	Modification of the fatty acid composition of oilseed rape seeds.	Wang *et al*. (2016)
Cassava	Cassava genome editing to increase resistance to cassava brown streak disease.	Odipio *et al*. (2020) Juma *et al*. (2022)
Peanuts	Gene editing for high-oleic acid phenotype with lower linoleic acid content.	Li *et al*. (2021) Yuan *et al*. (2019)
Oil palm	Gene-editing platform in oil palm targeting mutations.	Bahariah *et al*. (2023)
Chinese kale	Mutagenesis of homologous genes.	Sun *et al*. (2018)
Banana	Improving Cavendish banana using CRISPR/Cas9.	Naim *et al*. (2018)
Citrus	Gene editing for diesease management in citrus. CRISPR/Cas9 gene editing in Citrus using the YAO promoter.	Peng *et al*. (2017) Jia *et al*. (2019) Zhang *et al*. (2017)
Torenia fournieri	CRISPR/Cas9 system for modification of flower color.	Nishihara *et al*. (2018)
Japanese gentian	Molecular characterization of an anthocyanin-related glutathione S-transferase gene.	Tasaki *et al*. (2020)
Petunia hybrida	Genome editing by CRISPR-Cas9 technology.	Chopy *et al*. (2019)

create uncertainty for researchers and breeders who are developing genome-edited crops.

Another challenge is the potential unintended effects of genetic modifications and their impact on the environment and food safety. Although CRISPR-Cas technology allows for precise modifications of the plant genome, there is still a risk of off-target effects, where unintended modifications may occur at sites other than the intended target (Wang *et al*., 2022). This can lead to unintended changes in plant traits or gene expression, which could have unforeseen consequences for the environment or food safety.

There are also ethical considerations associated with the use of CRISPR-Cas technology in crops. For example, there are concerns about the potential impact of genetically modified crops on biodiversity and ecosystem services, and questions about the equitable distribution of benefits and risks associated with genetically modified crops as well as the potential impacts on small-scale farmers and indigenous communities (Wang *et al*., 2022). Furthermore, public perception and acceptance of genetically modified crops is also a challenge. Some consumers and advocacy groups are skeptical of genetically modified crops and may be resistant to accepting them, which could limit their adoption and commercialization (Ahmad *et al*., 2021 ; Eckerstorfer *et al*., 2019 ; Qaim, 2020). Addressing these challenges will require collaboration among scientists, policymakers, and stakeholders to ensure responsible use of CRISPR-Cas technology in crop improvement.

6. FUTURE PROSPECTS OF CRISPR-CAS-BASED GENOME EDITING IN CROPS

CRISPR-Cas technology has the potential to revolutionize crop improvement, and there are several potential applications of this technology in agriculture. One potential application is the improvement of yield and productivity. By introducing modifications that increase photosynthetic efficiency or reduce crop losses due to pests or diseases, CRISPR-Cas technology could help increase crop yields and food security. Another potential application is the improvement of stress tolerance in crops, such as drought or salt tolerance. By introducing modifications that increase the ability of crops to withstand adverse environmental conditions, CRISPR-Cas technology could help improve the resilience of crops to climate change and other environmental stresses. Moreover, the technology could also be used to introduce modifications that improve the nutritional content of crops. For example, researchers have used CRISPR-Cas to introduce modifications that increase the levels of beta-carotene in rice, which could help address vitamin A deficiency in developing countries (Wang *et al*., 2022).

However, realizing the full potential of CRISPR-Cas technology in crop improvement will require addressing the challenges and ethical considerations associated with this technology. As mentioned earlier, this will require collaboration among scientists, policymakers, and stakeholders to ensure responsible use of this technology in agriculture, as well as addressing public perceptions and concerns about genetically modified crops. Furthermore, there is a need for continued research and development to improve the efficiency and precision of CRISPR-Cas technology in crop improvement. Advances in genome sequencing, gene delivery systems, and other technologies could help improve the accuracy and specificity of genome editing in crops (Wang *et al*., 2022). In summary, CRISPR-Cas technology holds great promise for the future of crop improvement and has the potential to contribute to global food security, but there are also challenges and ethical considerations that need to be addressed. With continued research and responsible use, CRISPR-Cas technology could help address some of the most pressing challenges facing agriculture and food security in the coming decades.

CONCLUSION

CRISPR-Cas-based genome editing is a powerful tool for crop improvement with promising applications in agriculture (Wang *et al*., 2022). The technology enables the introduction of desirable traits such as increased yield, disease resistance, and stress tolerance through precise modifications of the plant genome (Li *et al*., 2016; Abdelrahman *et al*., 2021). However, there are also challenges and ethical considerations associated with the use of this technology in crops. The regulatory landscape for genome editing in crops is complex and varies by country, and there are concerns about the potential unintended effects of genetic modifications and their impact on the environment and food safety (Ahmad *et al*., 2021 ; Eckerstorfer *et al*., 2019 ; Qaim, 2020). Additionally, there are ethical considerations associated with the use of genetically modified crops, including questions about the equitable distribution of benefits and risks associated with genetically modified crops and their potential impacts on small-scale farmers and indigenous communities (Wang *et al*., 2022).

Despite these challenges, CRISPR-Cas technology holds great promise for the future of crop improvement. The technology could help increase crop yields, improve the resilience of crops to environmental stresses, and address nutritional deficiencies in developing countries (Wang *et al*., 2022). Addressing the challenges and ethical considerations associated with the use of CRISPR-Cas technology in crops will require collaboration among scientists, policymakers, and stakeholders to ensure responsible use of this technology in agriculture (Wang *et al*., 2022). Continued research and development to improve the efficiency and precision of CRISPR-Cas technology in crop improvement will also be crucial (Wang *et al*., 2022; Abdelrahman *et al*., 2021).

In a nut's shell, CRISPR-Cas-based genome editing has the potential to contribute to global food security and help address some of the most pressing challenges facing agriculture in the coming decades, but this potential needs to be realized in a responsible and ethical manner (Wang *et al*., 2022; Li *et al*., 2016; Abdelrahman *et al*., 2021; Ahmad *et al*., 2021 ; Eckerstorfer *et al*., 2019 ; Qaim, 2020).

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