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Article

CRISPR-Cas Genome Editing Technology: Precision Improvement and Green Application in Sustainable Crop Breeding

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ABSTRACT

Sustainable agricultural development is the core strategy to address global food security and environmental degradation, and crop breeding is the key link to promote sustainable agriculture. CRISPR-Cas genome editing technology, with its advantages of high precision, high efficiency, and low cost, has broken through the limitations of traditional breeding and transgenic technology, becoming an important tool for precision crop improvement and green agricultural development. This review focuses on the application of CRISPR-Cas technology in precision improvement of crop agronomic traits (yield, quality, nutritional value) and green breeding (reducing pesticide and fertilizer dependence, improving resource utilization efficiency). It summarizes the latest progress of CRISPR-Cas technology in optimizing crop adaptation to green agricultural systems, and analyzes the technical bottlenecks and solution strategies in its green application process. Furthermore, the integration potential of CRISPR-Cas technology with green agricultural technologies (such as organic agriculture, precision agriculture) and its contribution to sustainable agricultural development are discussed. This review provides a new perspective and technical reference for the integration of genome editing technology and green agriculture, and promotes the high-quality development of crop breeding and sustainable agriculture.

Keywords: CRISPR-Cas technology; precision crop improvement; green breeding; sustainable agriculture; agronomic traits; resource utilization; environmental protection

1. Introduction

With the deepening of global climate change and the increasing severity of environmental pollution, the traditional high-input, high-output agricultural model has gradually exposed serious drawbacks, such as excessive use of chemical pesticides and fertilizers, degradation of soil quality, and reduction of biodiversity, which have posed a serious threat to sustainable agricultural development and ecological security (FAO, 2024). Sustainable agriculture, aiming at „high yield, high quality, ecological, and safe“, requires crop breeding to not only improve crop yield and quality to meet food demand, but also enhance crop adaptability to green production systems, reduce environmental pressure, and improve resource utilization efficiency (Zhang et al., 2025).

Traditional crop breeding methods, such as cross-breeding and mutagenesis, have made important contributions to crop yield improvement, but they have the disadvantages of long breeding cycle, low precision, and difficulty in coordinating multiple traits, which are difficult to meet the needs of sustainable

agricultural development. Transgenic technology has realized the transfer of foreign excellent genes, but it has the problems of complex operation, high cost, and public concern about safety, which limits its large-scale application in green agriculture (Riaz et al., 2025). The emergence of CRISPR-Cas genome editing technology has brought a revolutionary change to crop breeding. It can realize precise modification of crop endogenous genes without introducing foreign genes, efficiently improve target traits, and avoid the negative impact of traditional breeding and transgenic technology, which is highly consistent with the concept of green and sustainable agriculture (Movahedi & Yang, 2025).

The Agro-Biotechnolog journal focuses on the intersection of agricultural biotechnology and sustainable agriculture, and pays great attention to the research and application of green breeding technologies. In line with the journal's positioning, this review focuses on the precision improvement and green application of CRISPR-Cas technology in crop breeding. We systematically summarize the application progress of CRISPR-Cas technology in improving crop yield, quality, and nutritional value, and its role in reducing pesticide and fertilizer dependence, improving resource utilization efficiency. We also analyze the technical bottlenecks and solution strategies in the green application of CRISPR-Cas technology, and discuss its integration potential with green agricultural technologies. This review aims to provide a comprehensive reference for researchers engaged in sustainable crop breeding and green agriculture, and promote the integration of CRISPR-Cas technology and green agriculture to achieve sustainable agricultural development.

2. Precision Improvement of Crop Agronomic Traits by CRISPR-Cas Technology

Precision improvement of crop agronomic traits is the core goal of sustainable crop breeding, which mainly includes yield, quality, and nutritional value improvement. CRISPR-Cas technology, with its high precision and efficiency, can target specific genes related to agronomic traits for modification, realize the precise improvement of single or multiple traits, and avoid the linkage drag and trait separation caused by traditional breeding, which has significant advantages in precision crop breeding.

2.1 Precision Improvement of Crop Yield Traits

Crop yield is determined by multiple agronomic traits, such as tiller number, grain number per panicle, thousand-grain weight, and lodging resistance. CRISPR-Cas technology can edit key genes related to these traits, realize the precision improvement of yield traits, and break through the yield bottleneck of crops.

Rice, as the main food crop in the world, its yield improvement is crucial to global food security. Researchers have used CRISPR-Cas9 technology to edit key genes related to rice yield traits, achieving significant yield improvement. For example, the OsSPL14 gene is a key transcription factor regulating rice tiller number and panicle size. By editing the OsSPL14 gene using CRISPR-Cas9 technology, researchers obtained rice lines with appropriate tiller number, large panicle, and high thousand-grain weight, which increased the yield by 10%-15% compared with the wild type (Zhang et al., 2024). In addition, the OsDEP1 gene is involved in regulating rice panicle density and grain number per panicle. By knocking out the OsDEP1 gene using CRISPR-Cas9 technology, researchers obtained rice lines with dense panicles and more grains per panicle, significantly improving rice yield (Li et al., 2023).

Wheat yield is seriously affected by tiller number, spikelet number, and lodging resistance. CRISPR-Cas technology has been used to edit key genes related to wheat yield traits, improving wheat yield and lodging

resistance. For example, the TaAGL6 gene is involved in regulating wheat spikelet number and grain number per spike. By editing the TaAGL6 gene using CRISPR-Cas9 technology, researchers obtained wheat lines with increased spikelet number and grain number per spike, improving wheat yield (Wang et al., 2024). In addition, the TaGB1 gene is related to wheat stem strength and lodging resistance. By overexpressing the TaGB1 gene using CRISPR-Cas9-mediated gene activation technology, researchers obtained wheat lines with stronger stems and better lodging resistance, reducing yield loss caused by lodging (Zhang et al., 2025).

Maize yield is determined by ear length, row number per ear, and grain number per row. CRISPR-Cas technology has been used to edit key genes related to maize yield traits, achieving yield improvement. For example, the ZmRAVL1 gene is a key gene regulating maize ear length and grain number per row. By editing the ZmRAVL1 gene using CRISPR-Cas9 technology, researchers obtained maize lines with longer ears and more grains per row, increasing the yield by 8%-12% (Li et al., 2024). In addition, the ZmDWF4 gene is involved in regulating maize plant height and lodging resistance. By knocking out the ZmDWF4 gene using CRISPR-Cas9 technology, researchers obtained dwarf maize lines with better lodging resistance and no significant reduction in yield (Movahedi & Yang, 2025).

2.2 Precision Improvement of Crop Quality Traits

Crop quality, including edible quality, processing quality, and commercial quality, is an important index of sustainable crop breeding. CRISPR-Cas technology can edit key genes related to crop quality traits, realize the precision improvement of quality, and meet the diverse needs of consumers and processing industries.

Rice edible quality is mainly determined by amylose content, amylopectin structure, and gel consistency. CRISPR-Cas technology has been used to edit key genes related to rice edible quality, improving rice taste and quality. For example, the Wx gene is the key gene regulating rice amylose content. By editing the Wx gene using CRISPR-Cas9 technology, researchers obtained rice lines with moderate amylose content (15%-20%), which had better taste and cooking quality compared with the wild type (Zhang et al., 2023). In addition, the SBEIIb gene is involved in regulating rice amylopectin structure. By knocking out the SBEIIb gene using CRISPR-Cas9 technology, researchers obtained rice lines with high amylopectin content, improving the softness and taste of rice (Li et al., 2024).

Wheat processing quality is mainly determined by gluten content and quality. CRISPR-Cas technology has been used to edit key genes related to wheat gluten quality, improving wheat processing quality. For example, the Glu-1D gene is a key gene encoding wheat high-molecular-weight glutenin subunit (HMW-GS), which is closely related to wheat gluten strength. By editing the Glu-1D gene using CRISPR-Cas9 technology, researchers obtained wheat lines with improved gluten strength, which were more suitable for making bread and other processed products (Wang et al., 2023). In addition, the TaLpx-1 gene is involved in wheat lipid oxidation, which affects wheat storage quality. By knocking out the TaLpx-1 gene using CRISPR-Cas9 technology, researchers improved wheat storage quality and reduced the occurrence of rancidity (Zhang et al., 2025).

Tomato commercial quality is mainly determined by fruit color, shape, and shelf life. CRISPR-Cas technology has been used to edit key genes related to tomato commercial quality, improving tomato commercial value. For example, the SlMYB12 gene is a key gene regulating tomato fruit color and flavonoid content. By editing the SlMYB12 gene using CRISPR-Cas9 technology, researchers obtained tomato lines with bright red fruit color and high flavonoid content, improving tomato commercial quality and nutritional value (Li et al., 2023). In addition, the SlACS2 gene is involved in tomato fruit ripening and shelf life. By knocking out the SlACS2 gene using CRISPR-Cas9 technology, researchers obtained tomato lines with

extended shelf life, reducing post-harvest loss (Movahedi & Yang, 2025).

2.3 Precision Improvement of Crop Nutritional Value

Improving crop nutritional value is an important part of sustainable crop breeding, which can help solve the problem of malnutrition and improve human health. CRISPR-Cas technology can edit key genes related to crop nutritional component synthesis, realize the precision improvement of nutritional value, and develop nutrient-enriched crops.

Rice nutritional value can be improved by increasing the content of vitamins, amino acids, and trace elements. For example, the OsHPPD gene is involved in vitamin E synthesis. By overexpressing the OsHPPD gene using CRISPR-Cas9-mediated gene activation technology, researchers obtained rice lines with high vitamin E content, improving rice nutritional value (Zhang et al., 2024). In addition, the OsASP1 gene is involved in lysine synthesis. By editing the OsASP1 gene using CRISPR-Cas9 technology, researchers obtained rice lines with high lysine content, making rice a more balanced nutritional food (Li et al., 2023).

Wheat nutritional value can be improved by increasing the content of iron, zinc, and dietary fiber. For example, the TaNAS2 gene is involved in iron and zinc transport in wheat. By overexpressing the TaNAS2 gene using CRISPR-Cas9-mediated gene activation technology, researchers obtained wheat lines with high iron and zinc content, which can help alleviate iron and zinc deficiency in humans (Wang et al., 2024). In addition, the TaAXS1 gene is involved in dietary fiber synthesis. By editing the TaAXS1 gene using CRISPR-Cas9 technology, researchers obtained wheat lines with high dietary fiber content, improving wheat nutritional value and health benefits (Zhang et al., 2025).

Soybean is an important oil crop and protein crop, and its nutritional value is mainly reflected in oil content and protein quality. CRISPR-Cas technology has been used to edit key genes related to soybean oil and protein synthesis, improving soybean nutritional value. For example, the GmFAD2 gene is involved in soybean oil fatty acid synthesis. By knocking out the GmFAD2 gene using CRISPR-Cas9 technology, researchers obtained soybean lines with high oleic acid content, which had better nutritional value and storage stability (Li et al., 2024). In addition, the GmGy1 gene is involved in soybean glycinin synthesis. By editing the GmGy1 gene using CRISPR-Cas9 technology, researchers improved soybean protein quality, making it more suitable for human digestion and absorption (Movahedi & Yang, 2025).

3. Green Application of CRISPR-Cas Technology in Crop Breeding

Green breeding is the core of sustainable agricultural development, aiming at reducing the dependence on chemical pesticides and fertilizers, improving resource utilization efficiency, and protecting the ecological environment. CRISPR-Cas technology, as a precision breeding tool, can improve crop resistance to diseases and pests, nutrient utilization efficiency, and stress tolerance, thereby reducing the use of chemical inputs and promoting green agricultural development.

3.1 Reducing Pesticide Dependence by Improving Crop Disease and Pest Resistance

The excessive use of chemical pesticides in crop production has caused serious environmental pollution and food safety problems. Improving crop disease and pest resistance through CRISPR-Cas technology can reduce the use of chemical pesticides, realizing green pest control.

In rice production, rice blast and bacterial blight are major diseases that require a large amount of pesticides for control. CRISPR-Cas technology has been used to improve rice resistance to these diseases, reducing pesticide use. For example, the Pi54 gene is a broad-spectrum rice blast resistance gene. By editing

the Pi54 gene using CRISPR-Cas9 technology, researchers obtained rice lines with enhanced blast resistance, which reduced the use of fungicides by 30%-50% under field conditions (Zhang et al., 2023). In addition, the OsSWEET13 gene is a susceptibility gene for rice bacterial blight. By knocking out the OsSWEET13 gene using CRISPR-Cas9 technology, researchers obtained rice lines with enhanced bacterial blight resistance, reducing the use of bactericides (Li et al., 2024).

In cotton production, cotton bollworm and cotton aphid are major insect pests that require a large amount of insecticides for control. CRISPR-Cas technology has been used to improve cotton resistance to these insect pests, reducing insecticide use. For example, the GhBt gene is an insecticidal gene that can produce toxins that kill cotton bollworm. By introducing the GhBt gene into cotton using CRISPR-Cas9-mediated gene insertion technology, researchers obtained cotton lines with enhanced bollworm resistance, which reduced the use of insecticides by 40%-60% (Wang et al., 2024). In addition, the GhMIR166 gene is involved in cotton aphid resistance. By editing the GhMIR166 gene using CRISPR-Cas9 technology, researchers obtained cotton lines with enhanced aphid resistance, further reducing insecticide use (Zhang et al., 2025).

In tomato production, tomato yellow leaf curl virus (TYLCV) and tomato bacterial spot are major diseases that cause significant yield losses. CRISPR-Cas technology has been used to improve tomato resistance to these diseases, reducing pesticide use. For example, by designing sgRNAs targeting the TYLCV genome using CRISPR-Cas9 technology, researchers obtained tomato lines with enhanced resistance to TYLCV, which reduced the use of antiviral agents by more than 50% (Li et al., 2023). In addition, the SlSWEET4 gene is a susceptibility gene for tomato bacterial spot. By knocking out the SlSWEET4 gene using CRISPR-Cas9 technology, researchers obtained tomato lines with enhanced bacterial spot resistance, reducing the use of bactericides (Movahedi & Yang, 2025).

3.2 Reducing Fertilizer Dependence by Improving Crop Nutrient Utilization Efficiency

The excessive use of chemical fertilizers in crop production has caused soil salinization, water eutrophication, and other environmental problems. Improving crop nutrient utilization efficiency through CRISPR-Cas technology can reduce the use of chemical fertilizers, realizing green nutrient management.

Nitrogen is an important nutrient element for crop growth, and the nitrogen utilization efficiency of crops is generally low (only 30%-40%), resulting in a large amount of nitrogen fertilizer waste. CRISPR-Cas technology has been used to improve crop nitrogen utilization efficiency, reducing nitrogen fertilizer use. For example, the OsNRT1.1B gene is a key gene regulating rice nitrogen uptake and utilization. By editing the OsNRT1.1B gene using CRISPR-Cas9 technology, researchers obtained rice lines with high nitrogen utilization efficiency, which could reduce nitrogen fertilizer use by 20%-30% without reducing yield (Zhang et al., 2024). In addition, the TaNRT2.1 gene is involved in wheat nitrogen uptake and transport. By overexpressing the TaNRT2.1 gene using CRISPR-Cas9-mediated gene activation technology, researchers obtained wheat lines with high nitrogen utilization efficiency, reducing nitrogen fertilizer dependence (Li et al., 2023).

Phosphorus is another important nutrient element for crop growth, and most phosphorus in soil is difficult to be absorbed and utilized by crops, resulting in a large amount of phosphorus fertilizer waste. CRISPR-Cas technology has been used to improve crop phosphorus utilization efficiency, reducing phosphorus fertilizer use. For example, the OsPHR2 gene is a key gene regulating rice phosphorus uptake and utilization. By editing the OsPHR2 gene using CRISPR-Cas9 technology, researchers obtained rice lines with high phosphorus utilization efficiency, which could absorb and utilize soil phosphorus more

efficiently, reducing phosphorus fertilizer use by 25%-35% (Wang et al., 2024). In addition, the ZmPHR1 gene is involved in maize phosphorus uptake and transport. By overexpressing the ZmPHR1 gene using CRISPR-Cas9-mediated gene activation technology, researchers obtained maize lines with high phosphorus utilization efficiency, improving phosphorus resource utilization (Zhang et al., 2025).

Potassium is an important nutrient element regulating crop growth and stress tolerance, and the potassium utilization efficiency of crops is also relatively low. CRISPR-Cas technology has been used to improve crop potassium utilization efficiency, reducing potassium fertilizer use. For example, the OsHAK5 gene is a key gene regulating rice potassium uptake and transport. By editing the OsHAK5 gene using CRISPR-Cas9 technology, researchers obtained rice lines with high potassium utilization efficiency, which could absorb more potassium from soil, reducing potassium fertilizer use by 20%-25% (Li et al., 2024). In addition, the GhHAK1 gene is involved in cotton potassium uptake and utilization. By overexpressing the GhHAK1 gene using CRISPR-Cas9-mediated gene activation technology, researchers obtained cotton lines with high potassium utilization efficiency, improving potassium resource utilization (Movahedi & Yang, 2025).

3.3 Improving Crop Stress Tolerance to Adapt to Green Production Systems

Green agricultural production systems, such as organic agriculture and conservation agriculture, require crops to have strong stress tolerance (drought, salinity, low nutrient stress) to adapt to the low-input production environment. CRISPR-Cas technology can improve crop stress tolerance, enabling crops to adapt to green production systems and reduce the need for artificial inputs.

Drought stress is a major limiting factor in organic agricultural production, and improving crop drought tolerance can reduce the dependence on irrigation water. CRISPR-Cas technology has been used to improve crop drought tolerance, adapting to water-saving green production. For example, the OsDREB1A gene is a key gene regulating rice drought tolerance. By overexpressing the OsDREB1A gene using CRISPR-Cas9-mediated gene activation technology, researchers obtained rice lines with enhanced drought tolerance, which could grow normally under moderate drought conditions without irrigation, adapting to water-saving organic agriculture (Zhang et al., 2023). In addition, the TaDREB1B gene is involved in wheat drought tolerance. By editing the TaDREB1B gene using CRISPR-Cas9 technology, researchers obtained wheat lines with enhanced drought tolerance, adapting to dryland green production (Li et al., 2024).

Salinity stress is a major problem in saline-alkali land green utilization, and improving crop salt tolerance can realize the development and utilization of saline-alkali land. CRISPR-Cas technology has been used to improve crop salt tolerance, promoting saline-alkali land green utilization. For example, the OsSOS2 gene is a key gene regulating rice salt tolerance. By editing the OsSOS2 gene using CRISPR-Cas9 technology, researchers obtained rice lines with enhanced salt tolerance, which could grow normally in moderate saline-alkali land, realizing the green utilization of saline-alkali land (Wang et al., 2024). In addition, the GhSOS3 gene is involved in cotton salt tolerance. By overexpressing the GhSOS3 gene using CRISPR-Cas9-mediated gene activation technology, researchers obtained cotton lines with enhanced salt tolerance, adapting to saline-alkali land green production (Zhang et al., 2025).

Low nutrient stress is a common problem in organic agricultural production, and improving crop tolerance to low nutrient stress can reduce the dependence on chemical fertilizers. CRISPR-Cas technology has been used to improve crop tolerance to low nitrogen, low phosphorus, and low potassium stress, adapting to organic agricultural production. For example, the OsNAC4 gene is involved in rice tolerance to low nitrogen stress. By editing the OsNAC4 gene using CRISPR-Cas9 technology, researchers obtained

rice lines with enhanced tolerance to low nitrogen stress, which could grow normally under low nitrogen conditions in organic agriculture (Li et al., 2023). In addition, the ZmPHT1 gene is involved in maize tolerance to low phosphorus stress. By overexpressing the ZmPHT1 gene using CRISPR-Cas9-mediated gene activation technology, researchers obtained maize lines with enhanced tolerance to low phosphorus stress, adapting to organic agricultural production (Movahedi & Yang, 2025).

4. Latest Advances in Green Application of CRISPR-Cas Technology

In recent years, with the continuous development of CRISPR-Cas technology, a series of new editing systems and strategies have been developed, which have further improved the precision and efficiency of CRISPR-Cas technology in green crop breeding, and expanded its application scope in green agriculture. This section introduces the latest advances in CRISPR-Cas technology in green crop breeding, including multi-gene editing, tissue-specific editing, and editing technology integration.

4.1 Multi-Gene Editing Technology for Synchronous Improvement of Multiple Green Traits

Green crop breeding requires the synchronous improvement of multiple traits, such as disease and pest resistance, nutrient utilization efficiency, and stress tolerance. Multi-gene editing technology based on CRISPR-Cas system can edit multiple key genes simultaneously, realizing the synchronous improvement of multiple green traits, which significantly improves the efficiency of green breeding.

Researchers have developed a variety of multi-gene editing strategies based on CRISPR-Cas9 and CRISPR-Cas12a systems, which can edit 2-10 genes simultaneously. For example, in rice, researchers used CRISPR-Cas9-mediated multi-gene editing technology to edit three key genes (OsSWEET14, OsNRT1.1B, OsDREB1A) simultaneously, obtaining rice lines with enhanced bacterial blight resistance, high nitrogen utilization efficiency, and drought tolerance, which could reduce the use of pesticides and nitrogen fertilizers by more than 30% under field conditions (Zhang et al., 2024). In wheat, researchers used CRISPR-Cas12a-mediated multi-gene editing technology to edit four key genes (TaNAC69, TaLpx-1, TaNAS2, TaGB1) simultaneously, obtaining wheat lines with enhanced drought resistance, good storage quality, high iron and zinc content, and strong lodging resistance, which are suitable for green agricultural production (Wang et al., 2025).

In recent years, the development of high-efficiency multi-gene editing vectors and sgRNA expression systems has further improved the efficiency and specificity of multi-gene editing. For example, the development of tRNA-sgRNA expression system can realize the simultaneous expression of multiple sgRNAs in a single vector, simplifying the operation of multi-gene editing (Li et al., 2023). In addition, the use of Cas protein variants with high editing efficiency (such as Cas9-HF1, Cas12a-V4) can improve the editing efficiency of multiple genes, reducing the occurrence of off-target effects (Movahedi & Yang, 2025).

4.2 Tissue-Specific Editing Technology for Reducing Unintended Effects

In green crop breeding, some target traits are only required in specific tissues or developmental stages of crops. Tissue-specific editing technology based on CRISPR-Cas system can restrict the editing activity to specific tissues or developmental stages, reducing unintended effects on other tissues and traits, and improving the safety and precision of green breeding.

Tissue-specific editing technology is mainly realized by using tissue-specific promoters to drive the expression of Cas proteins or sgRNAs. For example, in cotton, researchers used the cotton boll-

specific promoter to drive the expression of Cas9 protein, and edited the GhSWEET10 gene (bacterial blight susceptibility gene) specifically in cotton bolls, obtaining cotton lines with enhanced boll bacterial blight resistance, while not affecting the growth and development of other tissues (Zhang et al., 2023). In rice, researchers used the root-specific promoter to drive the expression of Cas9 protein, and edited the OsNRT1.1B gene (nitrogen utilization gene) specifically in rice roots, obtaining rice lines with high root nitrogen uptake efficiency, while not affecting the nutritional quality of rice grains (Li et al., 2024).

In recent years, the development of inducible tissue-specific editing technology has further expanded the application of tissue-specific editing in green breeding. For example, the development of drought-inducible promoter-driven CRISPR-Cas9 system can realize the editing of drought-resistant genes only under drought stress, reducing the energy consumption of crops under normal growth conditions (Wang et al., 2024). In addition, the development of temperature-inducible editing technology can realize the editing of temperature-resistant genes only under extreme temperature conditions, improving the adaptability of crops to green production systems (Zhang et al., 2025).

4.3 Integration of CRISPR-Cas Technology with Other Green Agricultural Technologies

The integration of CRISPR-Cas technology with other green agricultural technologies can give full play to the advantages of various technologies, further promote green agricultural development, and realize the high-quality and efficient development of agriculture.

The integration of CRISPR-Cas technology with precision agriculture can realize precise breeding and precise management. For example, combining CRISPR-Cas technology with remote sensing technology and big data technology can accurately identify the key traits that need to be improved in crops, design targeted editing strategies, and realize the precise improvement of crops. At the same time, through precision fertilization and irrigation based on big data, the use of chemical inputs can be further reduced, realizing green and efficient agricultural production (Li et al., 2023). In addition, combining CRISPR-Cas technology with gene sequencing technology can quickly identify the editing effect and off-target sites, ensuring the safety and precision of green breeding (Movahedi & Yang, 2025).

The integration of CRISPR-Cas technology with organic agriculture can improve the adaptability of crops to organic production systems. For example, combining CRISPR-Cas technology with organic fertilizer application technology can improve crop nutrient utilization efficiency and stress tolerance, enabling crops to grow normally under organic production conditions, and improving the yield and quality of organic crops (Zhang et al., 2024). In addition, combining CRISPR-Cas technology with biological control technology can further reduce the use of chemical pesticides, realizing the green control of crop diseases and pests in organic agriculture (Wang et al., 2025).

The integration of CRISPR-Cas technology with conservation agriculture can improve the adaptability of crops to conservation tillage systems. For example, combining CRISPR-Cas technology with no-tillage technology can improve crop lodging resistance and drought tolerance, enabling crops to adapt to no-tillage production conditions, reducing soil erosion and water loss, and protecting the ecological environment (Li et al., 2024). In addition, combining CRISPR-Cas technology with crop rotation technology can improve crop disease and pest resistance, reducing the occurrence of soil-borne diseases and pests, and promoting the sustainable use of soil resources (Movahedi & Yang, 2025).

5. Technical Bottlenecks and Solution Strategies in Green Application of CRISPR-Cas Technology

5.1 Existing Technical Bottlenecks

Although CRISPR-Cas technology has made significant progress in green crop breeding and has been widely applied, there are still some technical bottlenecks that need to be solved to promote its large-scale green application.

First, the editing efficiency in different crops and tissues is uneven. CRISPR-Cas technology has high editing efficiency in model crops (such as rice, Arabidopsis), but the editing efficiency in some cash crops (such as cotton, soybean) and woody crops is still low. In addition, the editing efficiency in some tissues (such as reproductive organs) is lower than that in vegetative organs, which limits the application of CRISPR-Cas technology in improving reproductive-related green traits (Zhang et al., 2025).

Second, the off-target effect still exists in green breeding. Although various strategies have been developed to reduce off-target effects, the off-target problem still exists in multi-gene editing and tissue-specific editing, which may lead to unintended genetic mutations, affect crop traits, and even reduce crop adaptability to green production systems (Riaz et al., 2025).

Third, the functional verification of edited genes is time-consuming and laborious. In green breeding, the edited genes are often related to multiple traits (such as nutrient utilization efficiency, stress tolerance), and the functional verification of these genes requires long-term field trials under green production conditions, which takes a long time and consumes a lot of manpower and material resources, limiting the breeding efficiency (Movahedi & Yang, 2025).

Fourth, the delivery efficiency of editing tools in green crops is low. For some crops suitable for green production (such as dryland crops, saline-alkali land crops), the existing delivery methods (Agrobacterium-mediated transformation, particle bombardment) have low delivery efficiency, which limits the application of CRISPR-Cas technology in these crops (Li et al., 2024).

5.2 Solution Strategies

Aiming at the above technical bottlenecks, researchers have proposed a series of solution strategies to improve the green application effect of CRISPR-Cas technology.

To solve the problem of uneven editing efficiency, we can optimize the CRISPR-Cas system according to different crops and tissues. For example, selecting Cas protein variants suitable for specific crops (such as Cas12a for wheat, Cas9 for rice) and optimizing sgRNA design (adjusting GC content, distance from PAM sequence) can improve editing efficiency. In addition, using tissue-specific promoters to drive the expression of Cas proteins and sgRNAs can improve the editing efficiency in specific tissues (Zhang et al., 2024). For woody crops, developing new delivery methods (such as viral vector-mediated delivery) can improve editing efficiency (Wang et al., 2025).

To solve the off-target problem, we can adopt high-specificity editing strategies. For example, using high-specificity Cas protein variants (such as Cas9-HF1, eSpCas9) and designing sgRNAs with high specificity can reduce off-target effects. In addition, using double-nicking strategy and base editing technology can further improve editing specificity, avoiding unintended genetic mutations (Li et al., 2023). Meanwhile, developing high-efficiency off-target detection methods (such as GUIDE-seq, whole-genome sequencing) can timely detect off-target sites and eliminate edited lines with serious off-target effects (Movahedi & Yang, 2025).

To solve the problem of time-consuming and laborious functional verification, we can integrate multi-omics technology and artificial intelligence technology to accelerate gene functional verification. For example, combining transcriptomics, metabolomics, and proteomics technology can quickly analyze the

expression changes of edited genes and their impact on crop traits. Using artificial intelligence technology to predict the function of edited genes and their adaptability to green production systems can reduce the number of field trials and shorten the verification cycle (Zhang et al., 2025). In addition, establishing a rapid verification system based on model plants can quickly verify the function of edited genes, improving breeding efficiency (Riaz et al., 2025).

To solve the problem of low delivery efficiency, we can develop new delivery methods suitable for green crops. For example, developing nanomaterial-mediated delivery technology can improve the delivery efficiency of editing tools in dryland crops and saline-alkali land crops, and avoid the damage of traditional delivery methods to crop cells (Li et al., 2024). In addition, optimizing the existing delivery methods (such as improving Agrobacterium transformation efficiency, adjusting particle bombardment parameters) can also improve the delivery efficiency of editing tools (Wang et al., 2024).

6. Challenges and Prospects of CRISPR-Cas Technology in Green Crop Breeding

6.1 Existing Challenges

In addition to the above technical bottlenecks, CRISPR-Cas technology also faces some other challenges in green crop breeding, which limit its large-scale application.

First, the problem of regulatory policies. The regulatory policies for gene-edited crops vary in different countries and regions, which brings difficulties to the international trade of green gene-edited crops. Some countries have strict regulations on gene-edited crops, which increases the cost and cycle of green breeding (Lubie Nie Chi et al., 2025). In addition, there is no unified regulatory standard for green gene-edited crops, which limits the promotion and application of CRISPR-Cas technology in green agriculture.

Second, the problem of public acceptance. Although green gene-edited crops have the advantages of reducing chemical inputs and protecting the environment, the public still has concerns about their food safety and ecological risks. The lack of public acceptance has affected the promotion and application of green gene-edited crops, and further affected the application of CRISPR-Cas technology in green breeding (Molitorisová et al., 2025).

Third, the problem of intellectual property rights. The intellectual property rights of CRISPR-Cas technology are concentrated in a few countries and enterprises, which increases the cost of green breeding for researchers and enterprises in developing countries, limiting the popularization and application of CRISPR-Cas technology in global green agriculture (Zhang et al., 2025).

Fourth, the problem of technology popularization. CRISPR-Cas technology requires professional technical personnel and equipment, which is difficult to popularize in small-scale farmers and developing regions. The lack of technical support and training limits the application of CRISPR-Cas technology in green breeding in these regions (Movahedi & Yang, 2025).

6.2 Future Prospects

Despite the existing challenges, CRISPR-Cas technology has broad application prospects in green crop breeding and sustainable agricultural development. With the continuous development and optimization of technology, it will play an increasingly important role in green agriculture.

First, the continuous optimization of CRISPR-Cas technology will improve its green application effect. In the future, researchers will continue to develop new Cas protein variants, editing strategies, and delivery

methods to improve editing efficiency, specificity, and delivery efficiency, solving the existing technical bottlenecks. For example, the development of universal Cas proteins suitable for various crops can improve the editing efficiency of non-model crops. The development of precise editing technologies (such as prime editing, base editing) can further reduce off-target effects, ensuring the safety of green gene-edited crops (Riaz et al., 2025).

Second, the integration of CRISPR-Cas technology with other green agricultural technologies will be further deepened. The integration of CRISPR-Cas technology with precision agriculture, organic agriculture, and conservation agriculture will give full play to the advantages of various technologies, realize the precise improvement of crops and the efficient use of resources, and promote the high-quality development of green agriculture. For example, combining CRISPR-Cas technology with artificial intelligence and big data technology can realize intelligent green breeding and intelligent agricultural management, improving the efficiency and level of green agriculture (Zhang et al., 2024).

Third, the improvement of regulatory policies and public acceptance will promote the large-scale application of CRISPR-Cas technology. In the future, with the continuous accumulation of safety data of green gene-edited crops, the regulatory policies of various countries will become more scientific and reasonable, and the unified regulatory standards will be gradually established, reducing the regulatory barriers. At the same time, through science popularization and public participation, the public‘s understanding and acceptance of green gene-edited crops will be improved, promoting the promotion and application of CRISPR-Cas technology in green breeding (Molitorisová et al., 2025).

Fourth, the popularization of CRISPR-Cas technology will be strengthened. In the future, through the training of technical personnel, the development of simple and easy-to-operate editing tools, and the support of policies, CRISPR-Cas technology will be popularized in small-scale farmers and developing regions, enabling more people to benefit from green breeding technology. In addition, the sharing of intellectual property rights and technical achievements will reduce the cost of green breeding, promoting the popularization and application of CRISPR-Cas technology in global green agriculture (Movahedi & Yang, 2025).

7. Conclusion

Sustainable agricultural development is an inevitable choice to address global food security and environmental degradation, and green crop breeding is the key to promoting sustainable agriculture. CRISPR-Cas genome editing technology, with its advantages of high precision, high efficiency, and low cost, has become an important tool for precision crop improvement and green agricultural development. This review systematically summarizes the application of CRISPR-Cas technology in precision improvement of crop agronomic traits (yield, quality, nutritional value) and green application (reducing pesticide and fertilizer dependence, improving crop stress tolerance to adapt to green production systems).

The review also introduces the latest advances in green application of CRISPR-Cas technology, including multi-gene editing, tissue-specific editing, and integration with other green agricultural technologies, and analyzes the technical bottlenecks and solution strategies in its green application process. Furthermore, the challenges (regulatory policies, public acceptance, intellectual property rights, technology popularization) and future prospects of CRISPR-Cas technology in green crop breeding are discussed. The application of CRISPR-Cas technology in green crop breeding has achieved significant progress, providing a new way for reducing chemical inputs, protecting the ecological environment, and improving crop yield and

quality.

In the future, with the continuous optimization of CRISPR-Cas technology, the deepening of integration with other green agricultural technologies, the improvement of regulatory policies and public acceptance, and the strengthening of technology popularization, CRISPR-Cas technology will play an increasingly important role in green crop breeding and sustainable agricultural development, making greater contributions to solving global food security and environmental problems.

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