 DOI: 10.19601/j.cnki.issn1943 - 9903.2025.06.009

Application and Prospects of CRISPR/Cas9 Genome Editing Technology in the Genetic Improvement of Fruit Trees

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Abstract CRISPR/Cas9 technology, a revolutionary gene-editing tool, has rapidly garnered attention in plant science owing to its simplicity, high editing efficiency, and cost-effectiveness. Besides, it offers unprecedented precision and efficiency in the genetic improvement of fruit trees. To date, this technology has been widely utilized to enhance fruit quality, improve stress resistance, and mediate growth and development. These applications demonstrate its immense potential in fruit tree breeding. Looking ahead, advancements in editing efficiency, expanded application scopes, comprehensive safety assessments, and improved regulatory frameworks are expected to further broaden the role of CRISPR/Cas9 in fruit tree breeding, thereby driving the fruit tree industry toward higher yield, superior quality, enhanced stress resilience, higher efficiency, and contributing to global food security and sustainable agricultural development. This article outlines the fundamental principles of CRISPR/Cas9 gene editing technology, its applications in plants (including fruit trees), and its pivotal role in genetic improvement and germplasm innovation.

Key words CRISPR/Cas9, Genome editing, Fruit trees, Genetic improvement, Germplasm enhancement

0 Introduction

Genome editing refers to the application of sequence-specific nucleases to modify an organism's genome at targeted sites, thereby altering its expected genomic sequence[1]. It relies on sequence-specific nucleases to introduce double-strand breaks (DSBs) at precise genomic locations, activating the cell's inherent repair mechanisms. In eukaryotes, DSBs are primarily repaired through two pathways, namely, non-homologous end joining (NHEJ) and homologous recombination (HR). The former frequently results in imperfect repairs, leading to various mutations, whereas the latter is a highly conserved mechanism that enables precise repair of DSBs using a homologous DNA sequence as a template. In plant genome editing, specific site mutations are predominantly induced through the NHEJ repair mechanism[2]. Since its discovery, CRISPR/Cas9 technology has revolutionized the field of plant genetic engineering. Indeed, this breakthrough has significantly enhanced the efficiency and precision of gene editing while dramatically reducing operational complexity and costs. In crop research, CRISPR/Cas9 has been widely adopted for diverse applications, including varietal improvement, resistance enhancement, architectural modification, and fruit quality optimization[3]. Similarly, CRISPR/Cas9 has achieved remarkable milestones in fruit tree research. For instance, targeted gene knockout has facilitated the development of early-flowering mutants, offering valuable tools for manipulating flowering time. Additionally, precision editing has substantially improved disease resistance in fruit trees, such as conferring resistance to citrus canker and banana *Fusarium* wilt[4-5], thereby boosting productivity and economic value. CRISPR/Cas9 has also demonstrated promise in modifying plant architecture. For example, dwarf phenotypes have been successfully generated in banana and pear varieties through specific gene knockouts, enhancing spatial efficiency and concurrently minimizing susceptibility to pests and diseases. Furthermore, fruit quality traits have been optimized through this technology, including the modulation of nutrient synthesis and aesthetic characteristics. Examples include the production of white-fleshed strawberries and hairless kiwifruit using this technology, which has significantly improved their market competitiveness.

As is well documented, fruit trees possess large genomes, high levels of genetic heterozygosity, and complex ploidy, due to an abundance of single nucleotide polymorphisms (SNPs). These factors partially hinder the genetic improvement of fruit trees. However, the completion of whole-genome sequencing for various fruit tree species and the simplification of sgRNA design have driven the implementation of CRISPR/Cas genome editing technology in numerous fruit trees. In turn, this has led to the development of edited strains with enhanced growth, quality, resistance, and other desirable traits. Despite these advancements, the application of the CRISPR/Cas9 system in fruit tree gene function research and varietal improvement remains comparatively underexplored compared with its use in model plants and annual crops. Besides, the majority of studies focused on establishing and optimizing genome editing systems, with limited progress in practical applications. With the successful sequencing of entire genomes for an increasing number of fruit tree species, the focus has shifted to

Received: March 12, 2025 Accepted: May 21, 2025

Supported by Scientific Research Start-up Fund for PhD. of Zhaoqing University (210046); the Zhaoqing Science and Technology Innovation Guidance Project (231017174162871, 241226220091239).

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analyzing gene functions and leveraging these findings for genetic improvement. Genome editing technology has emerged as a critical tool in this endeavor, particularly in the genetic enhancement of fruit trees. Among these technologies, CRISPR/Cas9 has garnered extensive attention since its introduction and has been rapidly adopted in plant research. In fruit tree research, the development of CRISPR/Cas9 ribonucleoprotein (RNP) gene editing technology, which avoids using exogenous DNA, has overcome the biosafety concerns associated with genetically modified fruit tree varieties. This breakthrough holds great relevance for creating novel fruit tree germplasm and advancing genetic improvement efforts.

1 Basic principles of CRISPR/Cas9 gene editing technology

Gene editing technology can be categorized into three main systems: Zinc Finger Nucleases (ZFNs), Transcription Activator-Like Effector Nucleases (TALENs), and Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) and their associated genes (CRISPR-associated, Cas)[6-7]. Notably, ZFNs and TALENs were the first systems to be applied for genome site editing[8-10]. These technologies not only enhance gene targeting efficiency but also enable targeted gene cleavage and knockout. However, their widespread adoption in laboratories has been obstructed by high experimental costs and technical challenges, consequently limiting their practical application[11]. In contrast, the CRISPR/Cas system is simpler, more versatile, and more cost-efficient[12].

The CRISPR/Cas system, primarily identified in bacteria and archaea, serves as a defense mechanism against viral and foreign DNA invasion. CRISPR structures are present in the genomes of over 40% of bacteria and 90% of archaea[13-15]. The CRISPR/Cas system comprises CRISPR sequence elements and the Cas gene family. CRISPR is composed of short, highly conserved repeat sequences (Repeats) interspersed with equally conserved spacer sequences (Spacers). Based on the core Cas proteins, the CRISPR/Cas system can be classified into three types: Type I, Type II, and Type III[16]. While Type I and Type III systems are multi-protein immune complexes, the Type II CRISPR/Cas system can cleave target sites using a single Cas protein[17]. Among these, CRISPR/Cas9 has emerged as the preferred gene editing tool owing to its low cost, high efficiency, and operational simplicity. The CRISPR/Cas9 system is primarily composed of the Cas9 protein and single-guide RNA (sgRNA). The target site typically comprises a 20-base sequence, followed by a Protospacer Adjacent Motif (PAM) sequence at the 3' end. Different PAM sequences correspond to different Cas9 proteins, with the Cas9 protein recognizing the PAM sequence NGG (where N represents any base). Upon the binding of the Cas9 protein to sgRNA, it first identifies the PAM sequence in the genome. Then it achieves precise localization of the target site through complementary base pairing between the sgRNA and the target DNA strand. Once localized, the Cas9 protein cleaves both strands of the target DNA, creating DSBs approximately three bases upstream of the PAM sequence. Subsequently, the cell activates its self-repair mechanisms, which may result in base insertions, deletions, or replacements at the DNA break site. This process eventually leads to gene mutations, enabling precise editing of the target gene.

2 Advantages and challenges of CRISPR/Cas9 technology

The CRISPR/Cas9 technology offers significant advantages for the genetic improvement of fruit trees, including high efficiency, precision, and broad applicability. However, its practical application faces several challenges, such as off-target effects and the persistence of residual foreign DNA. To mitigate off-target effects, a range of predictive tools have been developed, and highly specific Cas9 variants have been engineered. Moreover, the use of the CRISPR/Cas9 RNP system, which involves the direct delivery of Cas9 protein and sgRNA complexes, can circumvent the integration of exogenous DNA. This approach facilitates the generation of non-transgenic edited plants, thereby offering a safer alternative for fruit tree breeding.

3 Application of CRISPR/Cas9 gene editing technology in plants

In 2013, research teams from China, the United States, and the United Kingdom simultaneously reported the successful application of the CRISPR/Cas9 system for site-directed mutagenesis of endogenous genes in plants. Following these breakthroughs, the CRISPR/Cas9 system rapidly gained widespread adoption and demonstrated powerful gene-editing capabilities across a diverse range of plant species. In CRISPR/Cas9 research, phytoene dehydrogenase (PDS) is typically used as a model gene owing to its unique functional characteristics. Specifically, PDS is a key regulator of carotenoid synthesis in plants. When its expression is inhibited, carotenoid production is significantly reduced or completely halted, resulting in a distinct white phenotype. These easily observable and identifiable phenotypic alterations make PDS a preferred target gene for establishing CRISPR/Cas9 editing systems in various plants. Consequently, it has become an essential tool for exploring gene function and advancing genetic improvement in plants.

In 2013, Gao Caixia's team pioneered the use of CRISPR/Cas9 technology to selectively mutate the PDS gene in rice, achieving a mutation efficiency of 4% -9.4% and yielding rice albino homozygous mutants[18]. Around the same time, teams from Harvard University and the Sainsbury Laboratory successfully knocked out the PDS gene in Arabidopsis and tobacco using CRISPR/Cas9 technology[19-20]. Subsequently, researchers have reported the use of the CRISPR/Cas9 system to knock out the PDS gene in a wide range of species, including wheat[21], soybean[22], poplar[23], petunias[24], and watermelon[25]. Taken together, these studies highlight the versatility and effectiveness of CRISPR/Cas9 tech-

nology in plant genome editing.

4 Application of CRISPR/Cas9 gene editing technology in fruit trees

In recent years, the application of CRISPR/Cas9 gene editing technology in plant research has significantly expanded, with substantial progress in the field of fruit trees. The growing number of reports on the use of the CRISPR/Cas9 system in fruit trees highlights its potential to accelerate variety improvement, open new avenues for fruit tree breeding, and overcome the limitations of traditional transgenic approaches. This technology offers a more efficient and convenient approach to creating novel fruit tree germplasm resources.

In 2014, Jia Hongge and Wang Nian[26] pioneered the application of CRISPR/Cas9 in fruit trees by targeting and knocking out the endogenous PDS gene in sweet oranges, resulting in albino mutant plants. This breakthrough marked the beginning of CRISPR/Cas9's use in fruit tree research. In 2016, Nishitani[27] utilized the CRISPR/Cas9 system to mutate the PDS gene in apples, successfully knocking out both alleles and producing albino mutants. Meanwhile, Jia Honghe *et al.*[28] and Peng Aihong *et al.*[29] independently employed the CRISPR/Cas9 system to selectively knock out the citrus canker susceptibility gene CsLOB1, generating mutant plants with enhanced disease resistance compared to wild-type plants. Likewise, Nakajima *et al.*[30] applied the CRISPR/Cas9 system to knock out the *PDS* gene in grapes, yielding regenerated plants with white leaves. Ren Chong *et al.*[31] used CRISPR/Cas9 to knock out the L-idonate dehydrogenase (IdnDH) gene in the grape variety Chardonnay, thereby modulating the tartaric acid synthesis pathway and significantly improving wine quality. Malnoy *et al.*[32] introduced Cas9 protein and sgRNA complexes into grape and apple protoplasts via PEG-mediated transformation, successfully knocking out the grape powdery mildew susceptibility gene *MLO*-7 and the apple fire blight susceptibility genes *DlPM*-1, *DlPM*-2, and *DlPM*-4. Moreover, Hu Chunhua *et al.*[33] and Kaur *et al.*[34] independently utilized the CRISPR/Cas9 system to knock out the *PDS* gene in bananas, producing albino mutant plants. Subsequent studies have explored the application of gene editing technology in functional research, such as reducing banana plant height, extending shelf life, and enhancing disease resistance[35-38].

In 2018, Wang Zupeng *et al.*[39] successfully induced albino phenotype regenerated plants from G418-resistant callus tissue of kiwifruit using both the PTG/Cas9 mutagenesis frequency system and the CRISPR/Cas9 system. The results revealed that the PTG/Cas9 system achieved a mutation efficiency ten times higher than that of the traditional CRISPR/Cas9 system, highlighting its potential as a more powerful genome editing tool for kiwifruit. This study laid the groundwork for the application of the PTG/Cas9 system in other fruit trees. Beyond the aforementioned species, gene editing technology has also been applied in papaya[40-42], pear[43-44], chestnut[45], kiwifruit[46], blueberries[47], strawberry[48] and litchi[49]. Future research is anticipated to lead to additional reports on the application of gene editing technology in fruit trees, further advancing the field.

5 Prospects

The precision and efficiency of CRISPR/Cas9 gene editing technology offer unprecedented opportunities for fruit tree breeding, enabling targeted improvements in specific genes and the development of novel varieties with enhanced yield, superior quality, and greater stress resistance.

5.1 Increasing fruit tree yield CRISPR/Cas9 gene editing provides a cutting-edge and highly promising approach to improving fruit tree productivity. By precisely modifying key genes, this technology can optimize growth, stress resistance, and fruit quality. Key strategies include:

(i) Enhancing fruit development. Genes that mediate cell proliferation (*e. g.*, ARF transcription factors, EXPANSIN) to increase fruit cell number and size[50] can be edited. Floral inhibitors (*e. g.*, TFL1) may be inhibited, whilst flowering promoters (*e. g.*, FT) may be activated to extend flowering periods and promote flower bud formation[51]. Seed development genes (*e. g.*, AGAMOUS) can be modified to produce seedless fruits, thereby enhancing consumer appeal and market value[52].

(ii) Optimizing photosynthesis and nutrient allocation. Genes encoding key enzymes (*e. g.*, RUBISCO) can be edited to enhance light energy utilization. Additionally, sucrose transporters (*e. g.*, SWEET family) can be regulated to direct more photosynthetic products toward fruit development[53-54].

(iii) Improving stress resistance for stable yield. Stress-responsive genes (*e. g.*, DREB, P5CS) can be edited to enhance survival under drought, salinity, or extreme temperatures. Furthermore, susceptibility genes (*e. g.*, MLO for powdery mildew) can be disrupted, or immune pathways (*e. g.*, NPR1) can be activated to minimize yield losses[55-58].

(iv) Modulating hormone pathways. GA catabolism genes (*e. g.*, GA2ox) can be knocked out, or DELLA proteins (*e. g.*, Rht) can be inhibited to promote plant elongation and fruit growth. Moreover, *AUX/IAA* or *ARF* genes can be edited to influence fruit morphology and development[50,59-60].

(v) Regulating maturity and harvest period. Juvenility-related genes (*e. g.*, SPL family) can be edited to accelerate the transition to the reproductive stage. Additionally, ethylene synthesis/response genes (*e. g.*, ETR) can be edited to delay or promote maturation, thereby extending market availability[61-62].

(vi) Overcoming self-incompatibility. In cross-pollinating species (*e. g.*, apples, pears), editing S-RNase genes can disrupt self-incompatibility, thereby increasing fruit set rates[63]. CRISPR/Cas9-mediated precision editing holds revolutionary potential for fruit tree cultivation, addressing the limitations of conventional breeding and enabling high-yielding, stable, and sus-

tainable production.

5.2 Improving fruit quality CRISPR/Cas9 can be used to edit genes involved in sugar metabolism (*e.g.*, sucrose synthase genes) and acid metabolism (*e.g.*, malate dehydrogenase genes) to govern the sugar-acid ratio in fruits to optimize taste and flavor. Furthermore, editing key genes in pigment synthesis pathways, such as carotenoids and anthocyanins (*e.g.*, PDS, MYB transcription factors), can enhance fruit color and market value. Lastly, targeting genes related to ethylene synthesis or signal transduction (*e.g.*, ACS, ACO) can delay the ripening and aging processes of fruits, thereby extending shelf life and reducing post-harvest losses.

5.3 Developing disease- and pest-resistant germplasm Pests and diseases significantly influence fruit yield and quality during tree growth and development, posing major constraints to agricultural productivity. Traditional control methods, such as chemical pesticides, not only elevate production costs but also raise concerns regarding environmental pollution and food safety. Breeding resistant cultivars represents one of the most economical and sustainable solutions to these challenges. CRISPR/Cas9 technology offers a precise and reliable tool for enhancing disease and pest resistance in fruit trees, particularly against pathogens that are challenging to manage through conventional breeding. For instance, CRISPR/Cas9-mediated knockout of the citrus *CsLOB*1 gene conferred robust resistance to citrus canker[64]. Disruption of *CsWRKY*22[65] and DMR6[66] genes produced mutant plants with enhanced resistance to canker and Huanglongbing (citrus greening disease). In grapes, CRISPR/Cas9-targeted knockout of *WRKY*52 improved resistance to *Botrytis cinerea*[67]. Editing the banana BSV gene sequence resulted in 75% of regenerated plants exhibiting no disease symptoms under water stress[68]. Despite existing technical challenges, such as suboptimal delivery efficiency and regeneration protocols, further optimization of CRISPR/Cas9 systems is expected to accelerate the development of high-quality, disease-resistant fruit tree varieties in the future.

5.4 Optimizing growth and developmental traits Perennial fruit trees typically exhibit prolonged juvenile phases, which hinder the efficiency of conventional breeding programs for cultivar improvement. CRISPR/Cas9 technology has emerged as a cutting-edge tool in molecular breeding, allowing for the precise modification of key genes to enhance agronomic traits and optimize growth characteristics. For example, targeted editing of juvenility-related genes (such as the flowering regulators FT and LFY) can significantly shorten the time from juvenility to fruiting, thereby accelerating breeding cycles. Another notable example is the editing of pear, which induced flowering in tissue-cultured seedlings within one year[43]. Additionally, CRISPR/Cas9 can be employed to modify genes governing branching angle and canopy architecture, facilitating the development of compact or mechanization-compatible tree forms. Such innovations can enhance orchard management efficiency through high-density planting or streamlined mechanized operations.

5.5 Future development directions (i) Multi-gene editing: Future studies should focus on simultaneously editing multiple genes to achieve significant improvements in yield, quality, and stress resistance. (ii) Non-GMO breeding: Utilizing the CRISPR/Cas9 RNP system, techniques for editing without exogenous DNA integration can be developed, enabling the creation of non-GMO fruit tree varieties that meet market demands. (iii) Integration of precision breeding and big data: The combination of genomics, transcriptomics, and phenotypic data may enable precise identification of target genes, further enhancing the efficiency and success rate of breeding programs.

6 Discussion

Genome editing technology is one of the most effective methods for examining gene function. Compared to ZFN and TALEN, the CRISPR/Cas9 system is highly efficient, easy to operate, and widely applicable for plant gene function research. More importantly, the CRISPR/Cas9 system operates at the genome level, resulting in mutant plants with stable genetic traits. The complete knockout of key gene functions can be achieved through the design of suitable single-guide RNAs (sgRNAs), enabling the knockout of any gene to study its function. In fruit trees, the CRISPR/Cas9 system can be applied not only to functional genomics research but also to variety improvement, assisting in the swift and accurate generation of new fruit tree germplasm. Despite its widespread utilization, some limitations of the CRISPR/Cas9 system merit acknowledgment. For example, off-target effects have been reported during practical applications. The specificity of CRISPR/Cas9 targeted editing relies on a gRNA of approximately 20 nucleotides (nt). If the designed gRNA lacks high specificity for the target site, it may bind to multiple sites in the genome, leading to non-specific or mismatched editing, a phenomenon referred to as off-target effects. To mitigate this issue, specialized software, such as CRISPRseek[69], CCTop[70], and Cas OFFinder (http://www.rgenome.net/cas-offinder/), can be employed to predict potential off-target sites during target selection and gRNA design. In addition, for plants prone to off-target effects, selecting more efficient and specific Cas9 proteins[71-72] or optimizing the CRISPR/Cas9 system through modifications can aid in minimizing off-target occurrences.

At present, the majority of CRISPR/Cas9 systems in plants rely on Agrobacterium-mediated transformation to introduce exogenous genes into plant cells. However, this method results in transgenic plants, raising biosafety concerns and limiting public acceptance. Although CRISPR/Cas9-edited plants can be crossed with non-transgenic plants through conventional breeding methods such as hybridization and backcrossing, the possibility of exogenous DNA contamination cannot be completely excluded. For asexually propagated fruit trees such as bananas, conventional breeding methods are particularly challenging. In 2015, Woo *et al.*[73] pioneered a non-transgenic approach using PEG to introduce purified

Cas9 protein and sgRNA into protoplasts of Arabidopsis, tobacco, and rice, producing regenerated mutants free of exogenous DNA. This optimized CRISPR/Cas9 system provided a new pathway for acquiring non-transgenic mutants. In 2017, Liang Zhen *et al.*[74] used a gene gun to deliver the RNP complex (Cas9 protein and sgRNA) into immature wheat embryos, successfully editing two genes, TaGW2 and TaGASR7, and establishing the first exogenous DNA-free CRISPR-Cas9 RNP gene editing system in wheat. In 2023, the Yi Ganjun team integrated genome editing and gene-clearing elements on a single vector, achieving the deletion of introduced functional gene components following genome editing in bananas, thereby developing a transgene-free genome editing technology[75]. Broeck research team employed a dual approach, combining co-editing and chlorophyll screening systems, to modify banana *ALS* genes using the CBE system, successfully achieving transgene-free genome editing in bananas in 2025[76].

Noteworthily, in April 2016, the U. S. Department of Agriculture approved the use of CRISPR-Cas9 technology by Yang Yinong's laboratory at the University of Pennsylvania to develop non-browning white mushrooms and by DuPont Pioneer to create a new variety of waxy corn[77]. This approval signifies that genome-edited organisms can be cultivated and marketed without additional regulatory oversight. Other countries are anticipated to adopt similar policies, allowing the promotion and application of gene-edited organisms. While the application of gene editing technology in fruit trees remains in its early stages, the development of CRISPR-Cas9 RNP systems without exogenous DNA offers promising prospects for fruit tree breeding. For example, successful protoplast regeneration in bananas has been reported both domestically and internationally. By following Woo *et al.*'s method of PEG-mediated RNP delivery into banana protoplasts, non-transgenic germplasm could be obtained, thereby expanding breeding possibilities. Similarly, adopting Liang's gene gun approach to knock out susceptibility genes in fruit trees could potentially enhance disease resistance or even confer immunity. For instance, the "Zhongjiao 9" banana, developed by the Banana Genetic Improvement Center (Guangzhou Branch) of the Fruit Research Institute of Guangdong Academy of Agricultural Sciences, is completely immune to *Fusarium* wilt. If the susceptibility genes for *Fusarium* wilt can be identified in the "Zhongjiao 9" genome, CRISPR-Cas9 RNP technology could be used to knock out these genes, potentially creating non-transgenic plants with high resistance or immunity to the disease and significantly advancing the development of high-quality, high-yield, and disease-resistant banana germplasm.

Achieving high editing efficiency is a central objective in the application of the CRISPR/Cas9 system. Consequently, the ongoing optimization of genetic transformation and regeneration systems is paramount for enhancing the effectiveness of CRISPR/Cas9 in fruit trees. Earlier studies have documented that selecting appropriate promoters to co-express Cas9 and sgRNA can significantly boost editing efficiency, which is a key factor in achieving high-frequency mutations in fruit trees. Moreover, emerging gene-editing technologies offer new avenues to address the limitations of the CRISPR/Cas9 system. For instance, base editors enable precise single-nucleotide substitutions, whilst the CRISPR/Cpf1 system provides distinct advantages in multiplex gene editing and chromosomal fragment deletion[78-79]. As a result, base editing, prime editing, and the application of the CRISPR/Cpf1 system hold significant implications for fruit tree research. These advancements are expected to deliver more robust tools for gene function studies and variety improvement in fruit trees in the future. In summary, genome editing technology represents a feasible and effective approach for the genetic improvement of fruit trees. Its prospects are bright, and it is expected to be widely applied and promoted in the future.

7 Conclusions

The development of CRISPR/Cas9 gene editing technology has opened up a new path for the genetic improvement of fruit trees, ascribed to its high efficiency and precision, which are expected to exert a profound impact on the fruit tree industry. (i) CRISPR/Cas9 technology has been used to improve genetic transformation efficiency. Advances in optimizing off-target effects and enhancing editing accuracy will effectively improve the cultivation efficiency and quality of new fruit tree varieties. For example, through this technology, new varieties with robust stress resistance, high yield, and outstanding quality can be rapidly cultivated to fulfill market demand. (ii) The application of CRISPR/Cas9 technology will reduce the time and resource investment in traditional breeding processes, especially for fruit trees with longer generation cycles and high heterozygosity, where its advantages are more evident. Notably, CRISPR/Cas9 technology holds considerable potential for applications in enhancing fruit tree resistance, improving fruit quality, and regulating growth and development, contributing to the sustainable development of the fruit tree industry. For example, fruit trees cultivated through gene editing technology that are resistant to pests and diseases can not only reduce the reliance on pesticides but also lower production costs while also being more environmentally friendly. In terms of quality improvement, CRISPR/Cas9 technology can precisely regulate the synthesis of nutrients and the appearance quality of fruits, thereby enhancing consumer experience. (iii) With advancements in technology and the expansion of applications, CRISPR/Cas9 gene editing technology is expected to play a decisive role in fruit tree breeding and gene function research, promoting technological progress and economic growth. Therefore, CRISPR/Cas9 technology is anticipated to play an increasingly important role in the genetic improvement of fruit trees and have a profound impact on the long-term development of the fruit tree industry. Overall, the advent of CRISPR/Cas9 genome editing has revolutionized fruit tree breeding, marking a paradigm shift from traditional experience-driven methods to precision design-based strategies.

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