The Principle and Application of the CRISPR-Cas9 System in Plants

Haochuan Xu

Zhenhai High school of Ningbo, Ningbo, 315200, China

Abstract. Plant diseases are one of the critical factors affecting crop growth. They significantly threaten global food security, so cultivating excellent, disease-resistant varieties is the most effective strategy. CRISPR-Cas9 gene editing technology has gained significant attention since its discovery, as its simplicity and efficiency make it a necessary tool for molecular breeding. The paper examines the technical fundamentals and practical applications of CRISPR-Cas9 gene editing technology in the field of plant science. It systematically summarizes its use in enhancing plant resistance to fungi, bacteria, and viruses. The main advantages and disadvantages of this technology, along with its prospects and challenges, were discussed to provide references for future research.

Keywords: CRISPR-Cas9; Gene editing; Plants.

1. Introduction

Gene editing works by deleting, replacing, or inserting targeted gene segments in an organism's genome, and these modifications can produce specific biological functions and phenotypes, as well as breed new species. The DNA cleavage function in traditional gene editing tools depends on FokI enzymes, which operate inside zinc finger nucleases (ZFNs)[1] and transcription activator-like effector nucleases (TALENs)[2]. The third-generation genome editing technology, which has developed rapidly in recent years, consists of Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) and CRISPR-associated (Cas) 9 systems [3]. The third-generation genome editing technology has emerged as the dominant gene editing method because researchers have rapidly developed it to achieve high efficiency, operational convenience, and affordable costs. It achieves gene editing by targeting specific loci mainly through the ability of mutual recognition between DNA and RNA.

The social context of gene editing technology is deeply rooted in the dual drivers of humanity's quest to unravel the mysteries of life and address global challenges. Since the 21st century, the intensification of population aging, the escalation of chronic disease burdens, food security crises, and climate change threats have prompted the scientific community to seek transformative technological solutions. Climate change-induced expansion of crop diseases has been countered through CRISPR-mediated knockout of susceptibility genes, yielding blast-resistant rice and yellow leaf curl virus-resistant tomato varieties that maintain stable yields under extreme weather. The development of salt-tolerant wheat varieties offers cultivation potential for 2.3 billion hectares of global saline-alkali land. Such technological innovations not only alleviate food crises but also reduce pesticide usage, driving ecological agricultural transformation, especially in terms of plant breeding.

The artificial selection process in plant breeding relies on genetic material variation and recombination to achieve breeding objectives such as high yield, high quality, disease resistance, and pest resistance [4]. The lengthy selection cycle of conventional breeding techniques makes it challenging to meet rising breeding targets. Plant genetic engineering technology represents a form of biotechnology that emerged through the development of DNA recombination technology, genetic transformation technology, and tissue culture technology. As the core technology of traditional plant genetic engineering breeding, transgenic technology can transfer the target genes that determine the specific genetic attributes of a particular variety into the plant through specific techniques to modify the plant genome, realize genetic recombination, and cultivate new varieties with high yields, multi-resistant, and high quality[5]. However, transgenic technology cannot precisely modify target genes, and its practical application is associated with certain risks and controversies.

The innovations in gene editing technology help improve the lengthy and ineffective plant breeding process by simplifying hybridization and screening methods. Gene editing technology, through genetic modification methods, replaces natural genetic variation under specific conditions to create targeted and precise modifications for genes, thereby shortening both the breeding cycle and process and enhancing breeding efficiency. The CRISPR-Cas9 gene editing system has gained significant popularity among researchers, who have utilized it to edit plant genes over the past few years. The technology has also developed rapidly due to its wide range of applications, and targeted modification of plant genes has promoted the development of applications such as identifying plant gene functions and breeding new varieties [6]. This review examines several gene editing technologies, briefly outlines their basic working principles, and summarizes the applications of CRISPR-Cas9 gene editing systems in field crops, horticultural plants, and medicinal plants over the past few years. This review examines future trends in plant gene editing technology and its potential shortcomings, providing a reference for enhancing plant genetics and improving plant germplasm while advancing the development of plant breeding.

2. Overview of CRISPR Gene Editing Technology

Prokaryotic organisms utilize the CRISPR-Cas system as their natural immune defense mechanism, which comprises three essential elements: a CRISPR array, Cas proteins, and trans-activating crRNA (tracrRNA). The system detects DNA sequences that border target DNA sequences (protospacer adjacent motif, PAM) for immune defense operations. The CRISPR locus in the prokaryotic genome includes a leader sequence, repeat sequences, and spacer sequences. Cas genes, which encode Cas proteins, are either near CRISPR genes or elsewhere in the genome[7].

2.1 Acquisition of Highly Variable Spacer Regions in CRISPR

The proteins Cas1 and Cas2 recognize foreign DNA, enabling them to identify PAM (protospacer-adjacent motif) regions on the DNA. The DNA sequence that CRISPR selects for protospacer sequence acquisition occurs near the PAM region. After recognition, the Cas1/2 protein complex performs spacer sequence cleavage, and protospacer insertion occurs in the leader sequence downstream of the CRISPR array. The DNA break heals itself through the efficient non-homologous end joining (NHEJ) pathway to restore the double-strand break. A new spacer sequence is integrated into the CRISPR array within the genome at this point [8].

2.2 Expression of the CRISPR Array

The CRISPR array's leader sequence regulates the transcription process of the pre-CRISPR RNA. The transcription of tracrRNA follows that of pre-crRNA, and the two RNA molecules pair through complementary base-pairing. The pre-CRISPR RNA pairs with tracrRNA to form a double-stranded RNA that subsequently binds to the Cas9 protein to form a complex. The complex selects various spacer sequence RNAs according to the types of foreign DNA through RNase III, which facilitates the processing of pre-CRISPR RNA into short CRISPR RNAs (crRNAs) with one spacer RNA and repeat region fragments [9].

2.3 Activation of the CRISPR-Cas System

The crRNA, Cas9, and tracrRNA complex locates foreign DNA sequences and identifies protospacer sequences that match the crRNA sequence. The complex locates the PAM/protospacer site before unwinding the DNA helices to form an R-loop structure. During the DNA binding process, the crRNA finds its match on one DNA strand, but the other strand stays unattached. The precise Cas9 enzyme cuts DNA three nucleotides before the PAM site to produce a blunt-end cleavage product. The HNH domain of Cas9 performs DNA strand cutting at the crRNA-complementary position, and the RuvC domain cuts at the non-complementary strand. The DNA double-strand breaks (double-strand break or DSB) occur because of Cas9 activity, which suppresses foreign DNA expression[10].

2.4 Overview of the CRISPR-Cas9 system

Based on the structure and evolutionary relationship of Cas proteins, the current CRISPR/Cas system is divided into six types. The Cas proteins of type I, III, and IV systems are multi-subunit, while the Cas proteins of type II, V, and VI systems are single-subunit. Because the mechanism of the single-subunit CRISPR/Cas system is simple and easy to operate, most of the mature research and widely used single-subunit systems, such as the type II CRISPR/Cas9 system, the type V CRISPR/Cas12a (Cpf1) system, and the type VI CRISPR/Cas13a (C2c2) system. The CRISPR system utilizes a single guide RNA (sgRNA or gRNA) to target the target site. Then, a nuclease, such as Cas9, cuts the double-stranded nucleic acid in the target region, triggering the cell's endogenous DNA damage repair mechanism and ultimately editing the genetic information with the help of this mechanism. The CRISPR/Cas9 system plays a significant role in the editing process, such as knockdown, gene insertion, and base editing. The CRISPR/Cas9 system primarily relies on intracellular non-homologous end-joining (NHEJ), microhomology-mediated end-joining (MMEJ), or homologous recombination repair mechanisms to perform gene knockout, gene insertion, single-base editing, and other editing tools. (MMEJ) or homology-directed repair (HDR).

When the NHEJ system repairs double-strand breaks (DSBs), the Ku heterodimer recognizes and binds to the ends of the two breaks, which are then processed by DNA polymerase and nucleic acid exonuclease, and then end-joined to form a double-strand. When modifying the ends, the uncertainty in the binding order of the exonuclease and polymerase results in different scenarios, such as base deletion and insertion mutations at the repair site, which can lead to the inactivation of the target gene. It has also been reported that targeting the Cas9 protein to the target gene or to two sites on the chromosome results in deletion of the entire sequence within the target gene or on the chromosome. Microhomologous sequences used by MMEJ in the repair of double-stranded breaks are prone to cause deletion of the flanks of the original break, resulting in chromosomal abnormalities such as deletions, translocations, inversions, and other complex rearrangements. After the Cas9 protein causes a DNA double-strand break, HDR uses the homologous DNA sequence as a template for error-free repair in the presence of a homologous donor DNA, such as the sister chromatid of the damaged sequence. Precise gene insertion or single-base editing can be achieved with HDR if a homologous donor DNA (donor) is artificially provided that contains an exogenous gene or replaced nucleotides at a specific position.

3. Advantages of the CRISPR-Cas9 System for Genetic Engineering

3.1 Advantages over other gene editing tools

Compared with the first-generation ZFNs and second-generation TALENs gene editing systems, the CRISPR-Cas9 system has the following advantages. Compared with first-generation ZFNs and second-generation TALENs gene editing systems, the CRISPR-Cas9 system has the following benefits. First, it is simple to design and construct, widely applicable, and low-cost. ZFNs and TALENs require complex and costly assembly processes, whereas CRISPR-Cas9 technology is easier to implement, as it involves simply designing a 20 bp target sequence and combining it with straightforward methods. The guide RNAs (gRNAs) in CRISPR-Cas9 recognize PAM sequences (NGGs) in target genes, enabling Cas9 proteins to cleave the target genes and initiate the editing process. Almost all organisms have PAM sequences in their genomes, making CRISPR-Cas9 capable of precisely targeting and editing most genes.

In contrast, ZFNs and TALENs have more specific requirements and a narrower range of applications. Additionally, plant gene editing using CRISPR-Cas9 shows higher precision. While studies suggest that CRISPR-Cas9 has a higher off-target rate in large and complex genomes, such as the human genome, its off-target effects in plant research are relatively low[11]. The CRISPR-

Cas9 system enables users to edit multiple genes simultaneously when designing specific small interfering RNAs (siRNAs) for each target gene. Multiple sgRNAs and Cas9 coding sequences can be integrated into a single transformation vector to enable simulation.

3.2 Advantages over traditional genetic engineering

CRISPR-Cas9 technology offers several advantages over traditional transgenic methods, addressing many challenges associated with conventional crop breeding, such as long breeding cycles, reproductive isolation, and poor gene compatibility. First, unlike traditional transgenic techniques that can only reduce target gene expression at the RNA level with incomplete and unstable inhibition, CRISPR-Cas9 enables precise gene knockdown at the genomic DNA level. Second, CRISPR-Cas9 provides greater flexibility with multiple controllable gene editing sites. It allows the simultaneous editing of multiple loci and the precise regulation of gene mutations or insertions, thereby reducing the risks of adverse side effects associated with traditional methods, such as the disruption of critical endogenous genes. Third, CRISPR-Cas9 does not require the introduction of exogenous genes. Traditional transgenic methods often involve inserting exogenous genes, such as reporter or selection markers, which are stably inherited by offspring and can lead to public controversy. However, CRISPR-Cas9 technology enables breeders to separate gene editing from foreign gene insertion, allowing for the removal of unwanted foreign genes through selective breeding. The CRISPR-Cas9 system enables the precise removal of marker genes from previous transgenic processes, allowing genetically modified organisms to more closely resemble natural genetic variations and gain improved consumer acceptance [13].

3.3 Versatility and Scalability in Gene Regulation

The CRISPR-Cas9 system extends beyond gene knockout or replacement to enable multidimensional regulation of genes. By fusing catalytically inactive Cas9 (dCas9) with transcriptional activators (e.g., VP64) or repressors (e.g., KRAB), it can upregulate or downregulate target gene expression without DNA cleavage—a technique termed epigenome editing. For instance, in cancer Research, the dCas9-KRAB complex suppresses oncogene overexpression, while dCas9-VP64 activates tumor suppressor genes. Furthermore, Cas9 variants (e.g., Cas12a, Cas13a) broaden the scope: Cas12a recognizes flexible PAM sequences (e.g., TTTV), and Cas13a directly targets RNA molecules, enabling RNA-level editing and the clearance of viral RNA. This versatility positions CRISPR-Cas9 uniquely for both fundamental research (e.g., imaging chromatin dynamics) and clinical applications (e.g., gene expression modulation therapies), surpassing the singular cutting function of traditional tools.

4. Application of CRISPR-Cas9 technology in plants

4.1 Improving yield and quality

Yield and quality are key traits in agricultural production. Common indicators of yield include yield per unit area, plant size, the number of spikes, and the number of grains per spike. In contrast, indicators of quality concern include nutrient content, taste, and the safety of the crop. Recent studies have demonstrated that targeted mutation of specific genes using the CRISPR-Cas9 system can significantly enhance crop yield or improve its quality.

In terms of enhancing crop yield, the introduction of loss-of-function mutations in 11 Oryza sativa varieties into OsPDCD5 can increase plant height, optimize spike and grain shape, increase seed yield, and improve plant architecture. The construction of a weak promoter allele of the CLE gene successfully achieved quantitative variation in yield-related traits in Zea mays, resulting in improvements in several maize kernel yield-related traits. Knockdown of six BnSHP1 and two BnSHP2 homologs in Brassica napus resulted in increased resistance to pod shattering, thereby reducing oilseed yield losses. Mutation of BnaEOD1.A04 and BnaEOD1.C04 resulted in a significant increase in seed size and seed weight in the three edited lines, offering the possibility of breeding new germplasm for high-yielding oilseed rape[14].

In terms of crop quality improvement, the generation of ZmBADH2a and ZmBAGH2b single mutants and ZmBADH2a-ZmBAGH2b double mutants by editing in four selfed lines of maize indicated that these two genes were involved in the biosynthesis of the flavor substance 2-acetyl-1-pyrroline (2AP) in transgenic maize, and by editing both BADH2 genes simultaneously, researchers succeeded in creating a new crop quality by editing the two BADH2 genes. Genomes, the researchers succeeded in making the world's first maize germplasm containing the aroma substance 2AP. Human body micronutrient bioavailability decreases because of phytic acid, which causes nutritional deficiencies and reduced phytic acid content in seeds of the soybean (Glycinemax) GmIPK1 gene-edited lines. The vegetable oil fatty acid component erucic acid poses health risks. In contrast, three Brassica napus germplasm lines with high erucic acid (>30%) and high oil (>50%) developed low erucic acid Brassica napus germplasm through targeted mutation of two BnaFAE1 homologous gene copies. In cotton (Gossypium hirsutum), knocking down the GhFAD2 gene created a non-transgenic heterotetraploid cotton germplasm with a high oleic acid content.

4.2 Improving resistance

Plant resistance improvement demonstrates great promise through the use of CRISPR technology, which functions as a highly effective tool for gene editing. Plants now face an increasing number of severe stresses due to both global climate change and ecological pressures. Plants experience two types of stress: abiotic and biotic. Directed genetic changes in plants, such as those affecting disease resistance genes, insect resistance genes, drought resistance genes, and salt tolerance genes, create better crops that can withstand complex environmental conditions.

A CRISPR-Cas9 expression vector introduced through Agrobacterium-mediated transformation of soybean led to the Glyma.07g110300 gene mutant encodes UDP glycosyltransferase (UGT) and provides soybean resistance against leaf-chewing insects by modifying flavonoid content and related flavonoid biosynthesis gene expression patterns. Resistance. Modifying GmTAP1 in soybean plants improved resistance against P. sojae strains P231, P233, and P234. Binyameen et al. utilized the CRISPR-Cas9 Multigene Editing System through transient expression to edit CLCuD genes in cotton seedlings, resulting in virus-resistant cotton plants [15].

CRISPR-Cas9 technology is vital for enhancing plant defenses against environmental stress tribulations. The Oryza sativa OsbHLH024 gene editing mutant showed increased resistance to salt stress. Knocking down TaARR12 in a Triticum aestivum GW2 knockout mutant results in wheat exhibiting higher drought tolerance and grain yield. Editing ZmGA20ox3 to create a loss-of-function mutation enhances drought tolerance and increases maize plant height in maize seedlings. The rice wrky63 knockout mutant exhibits higher cold tolerance.

4.3 Gene function study

The field of life sciences places gene function studies in a vital spot because they help researchers understand organism development, environmental adaptation, and disease occurrence. Scientists utilize CRISPR-Cas9 technology to study genes accurately, thereby developing advanced methods for agricultural research.

Knockdown identified the PWA1 gene and yielded the rice tip2-1 mutant, which showed delayed chorion degradation, pollen abortion, and pollen wall defects, demonstrating that PWA1 is involved in pollen inner wall development through Os12BGlu38. CRISPR-Cas9 technology revealed that fungal pathogens secrete a subtilisin enzyme, interfering with rice immunity by degrading OsSGT1 proteins, thereby promoting infection. Thereby promoting infection. Mutant strains of maize with the YIGE1 gene exhibited reduced ear length and a lower number of kernels per row. Researchers created a quadruple BnaCOL9 mutation in canola using CRISPR-Cas9, which resulted in early flowering compared to wild-type plants. However, BnaCOL9 overexpression lines exhibited delayed flowering.

The BoALG10 knockout in ornamental kale (Brassica oleracea) mutant lines revealed an abnormal leaf margin structure, indicating that this gene functions crucially to preserve leaf margin form.

5. Potential Risks of CRISPR-Cas9 Technology Application

5.1 Off-target phenomenon

One of the most controversial criticisms of CRISPR-Cas9 gene editing technology is the off-target effect that occurs during its implementation. This phenomenon is primarily caused by mismatches between the sgRNA and the target DNA sequences, resulting in off-targeting. Off-targeting causes Cas9 to bind to and cleave unintended genomic loci, leading to unintended mutations and undesirable phenotypes in the plant [16]. The off-target effect leads to abnormal gene activation of disease susceptibility genes, while triggering chromosomal segment translocations and genomic instability, and causing unintended ecological impacts [17]. The CPC gene in newly generated transgenic T1 plants of Arabidopsis transgenic lines constructed using CRISPR-Cas9 showed an off-target mutation rate above 10%. Off-target mutation analyses of the T2 plants from six etc2 mutant lines revealed that CPC gene mutations occurred in more than 60% of plants on average. Studies to date have not comprehensively addressed off-target issues[18].

Multiple solutions exist to address the off-target effects of CRISPR-Cas9 technology. The functional analysis of SpCas9 led researchers to develop SpCas9-HF1 and enhanced SpCas9 (eSpCas9) as SpCas9 mutants, which aim to minimize Cas9/sgRNA non-specific DNA binding, particularly at non-target sites. Researchers can enhance sgRNA performance by modifying the Cas9 protein with adjustments in sgRNA length and chemical modifications. Delivering Cas9/sgRNA to target cells requires optimization for better results. The delivery of Cas9/sgRNA to target cells in cell culture requires plasmid transfection, ribonucleoprotein (RNP) electroporation, and viral transduction as commonly used methods to prevent off-target effects associated with prolonged expression. Ribonucleoprotein (RNP) electroporation and viral transduction can achieve transient peak expression of CRISPR-Cas9[19].

5.2 Gene conversion system constructs

CRISPR-Cas9 technology shows promising potential for enhancing the yield and quality of field and horticultural crops while improving their resistance through its applications. However, compared with field crops and horticultural plants, molecular biology research on medicinal plants is still in its infancy. First, a comprehensive gene conversion system has not yet been established in China, and the conversion systems of many key medicinal plants are not yet fully developed, which limits their application in exploration. Immature genetic transformation systems hinder progress, as stable and efficient platforms remain underdeveloped for most key species. Second, because the genomic information of medicinal plants in China is incomplete, and most of them have not undergone genome sequence analysis, gene editing using CRISPR-Cas9 technology is somewhat blind, making it difficult to assess their off-target effects. The scarcity of genomic data leads to uncertainties in editing. While transcriptomic studies have partially elucidated metabolic pathways in some species, the regulatory networks of core functional genes and non-coding regions remain poorly characterized. That forces reliance on homology-based target design, resulting in unpredictable off-target effects and reduced editing precision. There are several transformation methods for plant genome editing, such as Agrobacterium-mediated and PEG-mediated methods. However, Traditional tissue culture-dependent methods suffer from somatic variations induced by exogenous hormones, prolonged regeneration cycles, and low transformation efficiency, which limit the scalability of gene editing. Tissue culture regeneration methods have drawbacks, including the use of exogenous growth regulators that can lead to somatic cell clonal mutations and low transformation efficiency.

6. Optimization based on CRISPR-Cas9 technology

6.1 Base-Editing

Base-editing is a recent development based on the CRISPR-Cas system, which contains either an nCas9 (Cas9 nickase, Cas9 endonuclease) or dCas9 (dead Cas9, inactivated Cas9), and ssDNA (single-strand DNA) specific deaminase. This technology allows precise mutation of individual bases in cellular DNA without triggering DSBs, thus avoiding genomic instability and unpredictable results caused by DNA repair. These include the following types of base editors: Cytosine base editors (CBEs) that convert C-G to T-A, Adenine base editors (ABEs) that convert A-T to G-C, and Adenine base editors (ABEs) that convert A-T to G-C. T to G-C, Glycosylase base editors (GBEs) can convert C-G to G-C, Dual base editors (DBEs) can convert C-G to G-C, and Adenine base editors (ABEs) can convert A-T to G-C, Glycosylase base editors (GBEs) can convert C-G to G-C, and Dual base editors (DBEs) can convert C-G to G-C. Liang et al. developed a novel dual deaminase-mediated base editing system, AGBE, by integrating CGBE with ABE, which can simultaneously realize four types of base editing.

6.2 Prime-Editing

The core of base editing technology (Prime-Editing, abbreviated as PE technology) is a modified CRISPR-Cas9 composite system - it "binds" two key components together: One is pegRNA (primer-editing gRNA for short), and the other is a special Cas9 nuclease, specifically Cas9 (H840A mutant) fused with reverse transcriptase (Note: The H840A mutation enables Cas9 to only cut single strands of DNA, rather than the traditional double-strand breaks, which is one of the key features of PE technology. The precise modification capability of this system is mainly achieved through pegRNA. pegRNA itself is a "multifunctional carrier", consisting of three core parts: The first is the sgRNA (guide RNA) responsible for locating the target DNA, the second is the PBS (Primer binding Site) that helps the pegRNA bind to the DNA strand, and the third is the reverse transcription template carrying specific editing information. This feature of PE technology dramatically reduces the editing risk. Its working process can be broken down into "three steps": First, Cas9 (H840A) fused with reverse transcriptase precisely locates the target DNA and then cuts through one of the strands; Then, the reverse transcriptase will synthesize a DNA sequence carrying new genetic information according to the "instructions" of the reverse transcription template on the pegRNA. Finally, the cell's own DNA repair mechanism will "take over" and automatically integrate this new sequence into the genome, completing the entire editing process. However, at present, the application of PE technology in rice research has not yet overcome the "efficiency barrier": although it can achieve genomic modification in rice protoplasts (individual plant cells) and stable genetic lines (rice lines that can stably transmit traits), the overall editing efficiency has always been relatively low - this is also the problem that researchers are now focusing on solving.

6.3 Multigene-Editing

Multiplex genome editing (MGE) is a method to conduct coordinated genome modifications within multiple specific genomic targets. Through CRISPR-Cas9 technology, multiple editing loci become accessible by distancing Cas9 nuclease from its gRNA cohort. This method enables users to modify different locations simultaneously, thus creating new strains that contain multiple mutations. Multiple editing is the most significant advantage of the CRISPR/Cas9 system compared to TALENs, ZFNs, and other nucleases, as it enables the simultaneous expression of multiple guide RNAs (gRNAs) or Cas9 proteins. That significantly improves the efficiency and targeting range of gene editing or transcriptional regulation using the CRISPR/Cas9 system. The numerous editing makes this system extensively applied and developed in the field of bioengineering. Despite the maturation of current gRNA multi-expression strategies, the understanding of the mechanisms of gRNA multiplexing in the cell and its competitive role against Cas9 remains to be enhanced. The processing efficiency of multiple gRNA strategies (including Csy4, nuclease, and tRNA, among others) will play a key role in determining the theoretical upper limit on the number of gRNAs, and strategies for creating and processing long arrays will be optimized and enhanced in future studies.

7. Conclusion and prospects

This paper provides an in-depth exploration of the principles and applications of the CRISPR-Cas9 gene-editing technology in the realm of plant science. It begins with an introduction to the background of gene-editing technologies, highlighting the emergence and significance of the CRISPR-Cas9 system as a third-generation gene-editing tool, which is characterized by its efficiency and broad application potential.

The application of CRISPR-Cas9 technology for plant genetic engineering and breeding remains in its early stages despite recent theoretical and practical achievements because researchers need to improve several key aspects. First, establishing a diverse plant genetic transformation system. The lack of effective genetic transformation systems prevents many plants from performing large-scale research and applying CRISPR-Cas9 gene editing technology. Second, the mining of critical trait-determining genes. Completing whole genome sequencing for numerous plants, including crops, does not eliminate the need to identify genes related to essential traits.

The complete potential of CRISPR-Cas9 technology remains restricted for plant genetic engineering applications. Strategies for improving gene editing efficiency involve creating Cas9 enzyme variants with enhanced expression output, combined with the use of specific target area selection methods. The primary challenge lies in enhancing the precision of target locations. Scientists have developed strategies that reduce and eliminate unwanted effects in CRISPR-Cas9 through precise target site selection and design, sgRNA optimization, and modifications to Cas9. Today's research primarily applies CRISPR-Cas9 technology to target specific sites and knock out specific genes. The main advancement in CRISPR-Cas9 technology for plant genetic engineering improvement work lies in developing breakthroughs to transform the system and integrate functional genes at genomic target sites through HDR.

In conclusion, CRISPR-Cas9 gene editing technology is a crucial technical tool for plant gene function research, genetic breeding, and other research fields, as it offers several advantages over traditional transgenic technology, including ease of operation, speed, and affordability, as well as the absence of exogenous gene contamination following genetic editing. CRISPR-Cas9 gene editing technology holds a significant position that will expand across all plant research, including crop genetic engineering and breeding operations. A wide range of plant species requires extensive practical research to develop efficient gene editing systems for various crops. The development of plant gene editing technology necessitates an enhanced standardization system for plant genetic engineering, accompanied by continuous innovation in gene editing methods. Yet, its simple operation and excellent performance in trait improvement make it a vital tool for future plant genetics and molecular breeding.

References

[1] Carroll, D., Genome engineering with zinc-finger nucleases. Genetics, 2011. 188(4): p. 773-82.

[2] Bedell, V.M., et al., In vivo genome editing using a high-efficiency TALEN system. Nature, 2012. 491(7422): p. 114-8.

[3] Doudna, J.A. and E. Charpentier, Genome editing. The new frontier of genome engineering with CRISPR-Cas9. Science, 2014. 346(6213): p. 1258096.

[4] Collins, N.C., F. Tardieu, and R. Tuberosa, Quantitative trait loci and crop performance under abiotic stress: where do we stand? Plant Physiol, 2008. 147(2): p. 469-86.

[5] Liu, X., et al., Overexpression of the phosphatidylinositol synthase gene (ZmPIS) conferring drought stress tolerance by altering membrane lipid composition and increasing ABA synthesis in maize. Plant Cell Environ, 2013. 36(5): p. 1037-55.

[6] Sun, X., et al., Targeted mutagenesis in soybean using the CRISPR-Cas9 system. Sci Rep, 2015. 5: p. 10342.

[7] Sorek, R., C.M. Lawrence, and B. Wiedenheft, CRISPR-mediated adaptive immune systems in bacteria and archaea. Annu Rev Biochem, 2013. 82: p. 237-66.

[8] Bortesi, L. and R. Fischer, The CRISPR-Cas9 system for plant genome editing and beyond. Biotechnol Adv, 2015. 33(1): p. 41-52.

[9] Jinek, M., et al., A programmable dual-RNA-guided DNA endonuclease in adaptive bacterial immunity. Science, 2012. 337(6096): p. 816-21.

[10] Wiedenheft, B., S.H. Sternberg, and J.A. Doudna, RNA-guided genetic silencing systems in bacteria and archaea. Nature, 2012. 482(7385): p. 331-8.

[11] Shan, Q., et al., Genome editing in rice and wheat using the CRISPR-Cas system. Nat Protoc, 2014. 9(10): p. 2395-410.

[12] Xing, H.L., et al., A CRISPR-Cas9 toolkit for multiplex genome editing in plants. BMC Plant Biol, 2014. 14: p. 327.

[13] Xu, R.F., et al., Generation of inheritable and "transgene clean" targeted genome-modified rice in later generations using the CRISPR-Cas9 system. Sci Rep, 2015. 5: p. 11491.

[14] Liu, L., et al., Enhancing grain-yield-related traits by CRISPR-Cas9 promoter editing of maize CLE genes. Nat Plants, 2021. 7(3): p. 287-294.

[15] Binyameen, B., et al., Using Multiplexed CRISPR-Cas9 for Suppression of Cotton Leaf Curl Virus. Int J Mol Sci, 2021. 22(22).

[16] Cui, Y., et al., Review of CRISPR-Cas9 sgRNA Design Tools. Interdiscip Sci, 2018. 10(2): p. 455-465.

[17] Biswal, A.K., et al. CRISPR-mediated genome engineering to develop climate-smart rice: Challenges and opportunities. Semin Cell Dev Biol, 2019. 96: p. 100-106.

[18] Zhang, Q., et al., Potential high-frequency off-target mutagenesis induced by CRISPR-Cas9 in Arabidopsis and its prevention. Plant Mol Biol, 2018. 96(4-5): p. 445-456.

[19] Guo, C., et al., Off-target effects in CRISPR-Cas9 gene editing. Front Bioeng Biotechnol, 2023. 11: p. 1143157.

[20] Zhang, D., et al., Genome editing with the CRISPR-Cas system: an art, ethics and global regulatory perspective. Plant Biotechnol J, 2020. 18(8): p. 1651-1669.

[21] Vicencio, J., et al., Genome editing in animals with minimal PAM CRISPR-Cas9 enzymes. Nat Commun, 2022. 13(1): p. 2601.