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Advances in the Application of CRISPR-Cas9 Gene Editing Technology in Crops

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Abstract

CRISPR-Cas9 technology is based on the specificity of the CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) and Cas9 (CRISPR-associated proteins) systems, which allow for the precise editing of the DNA sequence of target genes. This technology is capable of targeting specific genes for editing, thereby enabling the regulation of important crop traits such as yield, disease resistance, and stress tolerance. The purpose of this paper is to summarize the progress of CRISPR-Cas9 gene editing technology in crop improvement. Firstly, the basic principle and operation process of gene editing technology are introduced. The application of this technology in crop genetic improvement and functional gene research is summarized. In addition, the challenges and prospects of this technology in crops are discussed. Although CRISPR-Cas9 technology has great potential for crop improvement, there are still some technical difficulties and ethical issues that need to be further explored and improved.

Keywords

CRISPR-Cas9, gene editing technology, crops, progress in application

1. Introduction

The importance of crop improvement has become more urgent with the growth of the global population and the effects of climate change. Conventional methods of crop improvement usually require a lot of time and resources, and progress is slow. Therefore, it is important to find new efficient and precise gene editing technologies to accelerate crop improvement. CRISPR-Cas9 gene editing technology is a revolutionary biotechnology that has attracted extensive attention and research in the field of gene editing in recent years. It utilizes the natural immune system of bacteria to perform precise genome editing by targeting specific gene sequences, providing an efficient, precise, and reproducible method for solving many biological problems. CRISPR-Cas9 technology has great potential for crop improvement. First, it can target specific genes for precise editing, enabling us to better understand gene function and regulatory mechanisms. Second, CRISPR-Cas9 technology is simple, efficient, and can be applied to a wide range of crops. This enables scientists to conduct gene editing experiments more quickly and accelerate the process of crop improvement. In addition, CRISPR-Cas9 technology can be used to improve important traits such as disease resistance, tolerance, and quality of crops by altering their genomes, providing a new way for the sustainable development of agriculture.

2. Technical Principles

2.1 Basic principles of CRISPR-Cas9 technology

CRISPR-Cas9 is a revolutionary gene editing tool that utilizes CRISPR sequences and Cas9 proteins in the natural immune system of bacteria and archaea to achieve precise editing of genomes. First, CRISPR sequences are part of the natural immune system of bacteria and archaea for recording information about foreign genome fragments. When a bacterium or archaea is infected with a foreign virus or plasmid, the CRISPR sequence records information about these foreign genome fragments and integrates them into its own genome. In this way, when the bacteria or archaea encounter

the same virus or plasmid again, the CRISPR sequence can recognize and destroy them through a specific mechanism. Secondly, the Cas9 protein is the core component of the CRISPR-Cas9 system with DNA-cleaving enzyme activity [1]. When the CRISPR sequence records information about foreign genomic fragments, the Cas9 protein binds to these fragments and shears them into small fragments by its cleaving enzyme activity. In this way, bacteria or archaea are able to destroy these foreign genome fragments and thus protect themselves from viruses or plasmids [2].

2.2 Import of RNA and Cas9 proteins to guide RNA and Cas9 proteins

In gene editing technology, importing RNA and Cas9 protein are two key steps. First, RNA import is the process of guide RNA synthesis followed by its introduction into cells. Guide RNA can be synthesized by chemical synthesis or in vitro transcription. Chemical synthesis is a common method in which the guide RNA can be synthesized using chemical reagents. In vitro transcription is another common method in which the guide RNA is synthesized in vitro using RNA polymerase. Once the guide RNA is synthesized, it needs to be introduced into the target cells. Transfection is one of the most commonly used methods to introduce the guide RNA directly into the cytoplasm using chemical reagents or electroporation. In addition to transfection, viral vectors can be used to introduce guide RNA into cells. Viral vectors are tools that allow exogenous genes to be introduced into the cell and expressed within the cell. The introduction and expression of guide RNA can be achieved by introducing the gene for the guide RNA into the viral vector and then infecting the target cell with the viral vector. Secondly, Cas9 protein is the core protein in the CRISPR-Cas9 system, which is responsible for recognizing and cleaving the target DNA. Similar to RNA import, the import of Cas9 protein can be achieved by transfection, electroporation, viral vectors, or gene transfection.

2.3 DNA cleavage and repair mechanisms during editing

DNA editing technology is a revolutionary biotechnology that can precisely modify the genome of an organism. Among them, CRISPR-Cas9 is one of the most commonly used DNA editing tools. In the CRISPR-Cas9 system, the Cas9 protein is able to recognize and bind to the target DNA sequence after forming a complex with the guide RNA. The Cas9 protein has two nuclease active sites that are able to cut both strands of the target DNA sequence. Once the target DNA sequence is cleaved, the cell initiates its own DNA repair mechanism. There are two main repair pathways: Non-Homologous End Joining (NHEJ) and Homology-Directed Repair (HDR). NHEJ is a common form of DNA repair, which repairs the cleavage site by directly joining the broken ends of DNA [3].

3. Crop improvement areas

3.1 Application of CRISPR-Cas9 technology in gene function studies

CRISPR-Cas9 technology has become a widely used tool for researchers because of its efficiency, precision, and flexibility in gene function studies. It can be used in studies of gene knockout, gene knock-in, gene mutation, and gene regulation. By designing appropriate guide RNAs, Cas9 proteins can accurately localize to target genes and trigger DNA double-stranded cleavage, thus realizing the editing of genes. For example, by knocking out a specific gene, researchers can learn about the gene's role in development, metabolism, or disease occurrence. A specific gene mutation is triggered to study the effect of the mutation on the organism. This helps to reveal the relationship between genes and phenotypes.

3.2 Application of CRISPR-Cas9 technology in the enhancement of resistance to pests and diseases

CRISPR-Cas9 technology can be used to improve pest and disease resistance in crops. By targeting specific disease and pest resistance-related genes for editing, plants can be enhanced against pathogens or pests. Disease resistance enhancement: researchers can use CRISPR-Cas9 technology to edit disease resistance-related genes in plants to enhance their resistance to pathogens. For example, by knocking out or mutating specific pathogen-recognizing genes, a plant's immune response to a pathogen can be enhanced. As another example, by knocking out or mutating specific pest-recognizing genes, the degree of plant infestation by pests can be reduced [4].

3.3 Application of CRISPR-Cas9 Technology in Adversity Tolerance Enhancement

CRISPR-Cas9 technology can be used to enhance the tolerance of crops to adverse environments, such as drought, salinity, and high temperature. By editing genes related to the response to adversity, the adaptability of plants to adversity can be improved. Drought Tolerance: through CRISPR-Cas9 technology, researchers can edit genes related to drought adversity response in plants to improve their drought tolerance. For example, by increasing the expression of drought-related genes in plants, the plant's ability to adapt to drought can be enhanced. And researchers have used CRISPR-Cas9 technology to edit genes in plants that are associated with saline and alkaline adversity response in order to increase the

plant's tolerance to saline and alkaline stress. For example, by reducing the expression of genes in plants that are sensitive to salt ions, plant adaptation to saline adversity can be enhanced.

3.4 Application of CRISPR-Cas9 Technology in Nutritional Quality Improvement

CRISPR-Cas9 technology can be used to improve the nutritional quality of crops. By editing genes related to nutrition, it is possible to increase the amount or improve the quality of specific nutrients in food. Vitamin increase: with CRISPR-Cas9 technology, researchers edit genes related to vitamin synthesis in plants to increase the amount of specific vitamins in plants. For example, by enhancing the expression of genes related to vitamin C synthesis in plants, the amount of vitamin C in plants can be increased. As another example, the nutritional value of proteins in plants can be improved by modulating the expression level or changing the structure of specific proteins in plants.

4. Examples of CRISPR/Cas9 system applications in crop variety improvement research

CRISPR/Cas9 gene editing technology has been successfully applied to a variety of model plants and crops, such as tobacco, Arabidopsis thaliana, rice, maize, wheat, sorghum, tomato, potato, and sweet orange, etc., and these examples of applications (Table 1) revealed properties such as gene expression, regulation of metabolic processes, mining of genes for resistance to pests and diseases and prolongation of the shelf time of vegetables [5]. Researchers have already utilized this technology for the manipulation and modification of crop genomes [6]. There are related studies using bioluminescent viral replicon to construct a set of heritable CRISPR/Cas9 gene editing systems, successfully synthesized tomato anthocyanin through the modification of HR, and inserted anthocyanin synthesis gene promoter and overexpression genes to accumulate anthocyanin, and finally obtained tomatoes with purple leaves. The use of transgenic crops to increase food production is an inevitable trend in the development of agricultural production. Compared with the traditional transfection method of Agrobacterium, the recombination ability of exogenous DNA has been improved 10-fold, thus breaking the bottleneck of introducing exogenous genes by recombination. At the same time, the transgenic plants also have good resistance to stress pests and diseases, as well as increased yield and improved quality. Using CRISPR-Cas9 multi-gene editing technology, Cai et al. [7] successfully synthesized a mutant with reduced root hairs in soybean, and this achievement provides strong support for the subsequent research on the functional genome of soybean roots.

Table 1. Cases of improved traits using genome editing

species	target gene	Editorial efficiency%	change in temperament	Transformation methods
corn	Zm PK	13.1	Phytate synthesis	Agrobacterium-mediated transformation
corn	Zm HKT1	-	Ion Transportation	Agrobacterium-mediated transformation
non-tobacco	NtPDS	82.0	yellowing of leaves	Agrobacterium-mediated transformation
non-tobacco	NtPDR6	88.0	branching	Agrobacterium-mediated transformation
maize	Os SW EETla	70.0	Bacterial leaf blight resistance	Agrobacterium-mediated transformation
maize	OsBEL	2.0-16.0	Sensitivity to herbicides	Agrobacterium-mediated transformation
lycopene	SIS1AGO7	48.0	Blade shape	Agrobacterium-mediated transformation
lycopene	SIRNN	25.0~100.0	Fruit ripening time	Agrobacterium-mediated transformation
lycopene	SLANT1	67.0	Tissue overexpression of anthocyanins	binary virus
potatoes	St AA2	50.0	Petiole and branch deformation	Petiole and branch deformation
soybeans	Gm DD1	0~14.0	DNA methyltransfer plays	genetic gunnery
soybeans	Bar Gm FE2, Gm SHR	10.0~93.3	Reduce the number of root hairs	Agrobacterium-mediated transformation
maize	TaMIO-Al	5.6	powdery mildew resistance	genetic gunnery

5. Strengths and challenges

CRISPR-Cas9 technology has the following advantages in terms of precision, efficiency, and cost:

(1) Precision: CRISPR-Cas9 technology can accurately locate and edit target gene sequences, making gene editing more precise and controllable.

(2) Efficiency: Compared with traditional gene editing technology, CRISPR-Cas9 technology has higher editing efficiency and can realize the editing of target genes more quickly.

(3) Cost: CRISPR-Cas9 technology is relatively simpler and more economical, and does not require complex equipment and expensive reagents, reducing the cost of gene editing.

Challenges of CRISPR-Cas9 technology:

(1) Uncertain Shear Efficiency: CRISPR-Cas9 technology may have uncertain shear efficiency when editing genes. Sometimes, the editing efficiency may be low and several attempts are required to get the desired result.

(2) Non-specific shearing: when editing a target gene, CRISPR-Cas9 technology may undergo non-specific shearing, i.e., shearing to a non-target gene, resulting in unexpected gene alterations.

(3) Potential unintended consequences: Gene editing may lead to unintended consequences, such as the introduction of new mutations or unforeseen genetic changes. These consequences may negatively affect the growth and development of crops and require strict safety assessment and monitoring.

6. Future outlook

As the research of CRISPR-Cas9 technology continues to deepen, researchers will further improve the editing efficiency and accuracy, making gene editing more effective and reliable. Moreover, CRISPR-Cas9 technology can edit multiple genes at the same time, which will provide more possibilities for crop improvement. Simultaneous editing of multiple genes can lead to more complex trait improvement and adaptation enhancement. In addition to CRISPR-Cas9 technology, other genome editing technologies are also evolving, such as CRISPR-Cas12 and CRISPR-Cas13, which have the potential to further improve the efficiency and accuracy of gene editing. CRISPR-Cas9 technology is capable of realizing non-transgenic improvement, i.e., editing genes without importing exogenous genes. It is conducive to allaying the public's concerns about GM technology and promoting the widespread dissemination of crop improvement. In addition to crop improvement, CRISPR-Cas9 technology can also be used in animal breeding, disease treatment, and biofuel production. With the development of technology, CRISPR-Cas9 technology may show its potential in more fields.

7. Conclusion

In summary, by utilizing the DNA self-repair mechanism, the CRISPR/Cas9 system is able to modify genes to specific sites, thus avoiding the problems of low efficiency in traditional breeding and uncertainty in transgenic technology, and providing a new way of thinking about crop variety improvement. The technology is characterized by high polymorphism and controllability, which provides a new way for GMO safety evaluation. With the continuous and in-depth research of modern bionics and bioinformatics, more and more varieties can be conveniently mined for valuable genetic information and complete plant genome sequencing. Therefore, the regulation of gene expression and metabolic processes is realized through gene editing technology, such as screening plant chromosome ploidy, inserting target gene sequences or deleting genomic regions, etc., to modify the lines that need to be improved, in order to obtain transgenic lines that contain the desired alleles and have excellent line characteristics.

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