Proceedings of the 3rd International Conference on Modern Medicine and Global Health
DOI: 10.54254/2753-8818/2024.KA19746

Research Progress of CRISPR Technology in Plant Abiotic Stress

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Abstract: In recent years, the agricultural sector has faced immense challenges due to prolonged droughts, excessive soil salinity, and extreme temperature fluctuations, which have collectively stunted crop growth and significantly reduced yields. Such environmental stressors not only impact agricultural productivity but also pose a substantial threat to global food security. To address these challenges, advancements in gene editing have introduced CRISPR (clusters of regularly interspaced short palindromic repeats) tools, with CRISPR-Cas9 emerging as a particularly powerful and widely adopted technology. This tool offers high precision, efficiency, and cost-effectiveness, enabling targeted genetic modifications that can enhance crop resilience and facilitate the development of superior varieties. This paper discusses the fundamental principles and unique characteristics of CRISPR-Cas9 technology, providing a comprehensive summary of its applications in strengthening crop resistance to various abiotic stresses, including drought, salinity, and extreme temperatures. Additionally, it addresses the technology's limitations, outlining the areas that require further refinement to optimize its agricultural impact and broaden its applicability.

Keywords: CRISPR, CRISPR-Cas9, abiotic stress, crop

1. Introduction

Abiotic stress refers to a range of environmental factors including unsuitable temperature, drought, salinity and other factors that are not suitable for plant survival, growth and development. In recent years, with the rapid growth of the global population, food consumption has risen sharply. Some experts predict that the global population will peak at over 10.4 billion in 2086. However, in terms of current agricultural production capacity, we cannot guarantee the synchronized growth of some important crops, such as wheat, corn, soybean and maize. At the same time, plants are more vulnerable to different abiotic and biotic stresses due to climate change and human activities. The global average temperature is increasing rapidly. In this scenario, abiotic and biotic stresses (partly including plant diseases and pests) are the main reasons for the drastic decrease in food production. The above situation has led to the problem of world food security. This has prompted the search for other new ways to improve the situation.

Thus, a new era of gene editing has arrived. In 2013, after the tireless efforts of the duo of Jennifer Doudna and Emmanuelle Charpentier, a brand new method of genome editing, Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR), a new genome editing method, has appeared in the public eye. CRISPR has become the technology of choice for the scientific community due to its precision, efficiency, and simplicity compared to zinc finger nucleases (ZFN) and transcription activator-like effector nucleases (TALEN) [1]. CRISPR-Cas9 is simpler to design than ZFN and TALEN. In addition, CRISPR-Cas9 has low production cost, is not restricted by methylation, and can edit multiple genes at the same time. CRISPR-Cas9 consists of a specific sgRNA and a non-specific endonuclease, Cas9. Gene editing systems mediated by it can respond to these pressures by knocking out the relevant genes. This technology also helps to functionally characterize genes associated with various biotic and abiotic stresses in target organisms. Many studies have shown that the CRISPR/Cas system has been applied to several plant species to improve their tolerance to major abiotic stresses. Among the most studied crops are rice and Arabidopsis.

However, there are still some challenges with this technology that require further research. The main issues are off-target effects and the limited scope of genomic targeting due to PAM sequence restrictions [2]. In addition, CRISPR has not yet been fully accepted by the public, and how to disseminate it as it matures is also a key issue. This paper focuses on the application of CRISPR in helping crops resist drought, salinity and extreme temperature conditions.

2. Brief description of CRISPR-CAS9 technology

2.1. Overview of CRISPR technology

CRISPR, known as regularly clustered interspaced short palindromic repeats, is an acquired immune system discovered from bacteria that can integrate viral genetic sequences into bacterial genomes. CRISPR-CAS9 consists of gRNAs that guide ribonucleic acid (including tracer RNAs and CRISPR RNAs) and the nucleic acid endonuclease CAS9 (an endonuclease that contains RuvC and HNH structural domains that are responsible for cutting the destination and non-destination strands of the target gene, respectively) consist of [3]. The cleavage site is located 3-8 nt upstream of the PAM.

First, the bacteria detect the exogenous DNA injected by the virus and allow the integration (adaptation) of short fragments of exogenous DNA into the CRISPR motif, and then, (crRNA biogenesis) transcribes and shears the CRISPR gene fragments into short RNA fragments, which together with the proteins encoded by the CAS genes, form complexes (complexes). These complexes will utilize the RNA fragments of CRISPR to base pair with the DNA molecules of the virus. CAS9 generates double-strand breaks (DSBs) at target sites in the genome, causing non-homologous end joining (NHEJ) and homology repair (HDR) mechanisms [4], leading to insertions, deletions and substitutions of the target gene sequences, resulting in alterations in genomic information. Among them, sgRNAs can be designed in advance to guide CAS9 to recognize target sequences in relevant genes for localization.

2.2. Base editors

Since CRISPR-Cas9 technology cannot be used for base conversion of genes, it is only suitable for knockout or knock-in of genes. Base editor is a genome editing tool that can accurately change nucleotides without DSB. With the help of CRISPR-Cas9 system to localize the target site, and then realize the precise mutation of the target base through the fusion of base deaminase, it can improve the efficiency of the fixed-point mutation. Depending on the deaminase, base editors can be categorized into single-base editors and multi-base editors, and single-base editors can be divided into adenine base editors (ABE) and cytosine base editors (CBE), whereas ABE is based on the core of adenine deaminase, which is composed of the combination of sgRNA and nCas9 proteins, and can efficiently convert the base pairs of A-T to G-C. CBE is based on the core of cytosine deaminase, which is composed of sgRNA and nCas9 protein. Cytosine deaminase as the core, also including sgRNA, nicking Cas9 (nCas9) protein, uracil glycosylase inhibitor (UGI), which can realize the efficient conversion of C-G to T-A base pairs Dual base editor can induce C to T and A to G mutations at the target site simultaneously through the guidance of gRNA.

Base editing technology is a powerful and convenient tool in crop breeding, which solves the problems of randomness and low efficiency of traditional mutation breeding, and also enhances the broad-spectrum disease resistance of crops by editing disease-sensitive genes, which is helpful to cultivate high-yield and disease-resistant new crop varieties.

2.3. CRISPR-Cas9 system design ideas

The vector of the CRISPR-Cas9 system is re-designed from the successfully edited crop. The T-DNA insertion region of CRISPR-Cas9 vector can be divided into three parts: Cas9 protein expression cassette, sgRNA expression cassette, and screening gene expression cassette [5]. Among them, Cas9 protein is mainly driven by RNA polymerase II promoter, while sgRNA is mainly driven by polymerase III promoter. Phytoene desaturase (PDS), negatively regulated genes for plant disease resistance, and genes mutated to cause abnormal plant growth were used as targets. Depending on the number of targets, there are differences in the construction methods of different numbers of target vectors. In the construction of single-target vectors, sgRNAs are usually ligated to the sticky ends generated by Bsa I restriction endonuclease cleavage through Golden Gate ligation, while multiple targets are introduced into plant expression vectors by assembling them into a cassette gene. The method of vector delivery varies among species, with Agrobacterium-mediated transfer into highly regenerative explants of young leaves, cotyledons, or embryonic cell cultures being the predominant approach for most crops.

To improve the efficiency of gene editing, the selection of an appropriate promoter is crucial. The polymerase III promoters U3 and U6 are usually selected to drive sgRNA expression. sgRNA GC content as well as secondary structure are the two major keys to gene editing efficiency. In addition to sgRNA selection, efficient transformation vectors can be realized by vector delivery methods to improve the effectiveness of gene editing. Vector delivery is categorized into direct and indirect delivery methods, and direct methods, including peg-mediated and shelling-mediated delivery of CRISPR/Cas9 RNPs, including traditional Agrobacterium infections and rhizobial Agrobacterium infections are indirect transformation methods, which are less efficient than direct transformation. In recent years, nanoparticles (e.g., carbon nanotubes) have been used as carriers with broad prospects.

3. CIRSPR in crop resistance to abiotic stresses

3.1. Drought

Under drought stress, abscisic acid (ABA) content in plants will undergo a significant increase, mainly by affecting the synthesis pathway and metabolic regulation of ABA to control ABA content. ABA on the one hand, in order to inhibit water loss can promote stomatal closure and inhibit stomatal opening, on the other hand, in response to stress it activate a series of stress-resistant gene expressions. In rice, ABA signaling mainly relies on stress-activated protein kinase (SAPK2), a mediator that regulates drought stress by closing stomata, increasing the synthesis of compatible solutes, and inducing the expression of ROS scavengers. Under drought conditions, SAPK2 made the mutant show insensitivity to ABA by regulating the expression of polyethylene glycol. The CRISPR-Cas9 system can be utilized for the identification process of its loss of function due to mutation on the third exon. In addition, the OsEBP89 gene is involved in drought tolerance in rice by increasing ROS scavenging and proline accumulation in cells under drought stress. Multiple gene expressions that can be induced by this gene can be knocked down by the CRISPR-Cas9 system as a way to attenuate the adverse effects on the plant. Mitogen-activated protein kinase (MAPK) in crops is closely related to drought stress [6], and activation and regulation of the cascade pathway of MAPK has an important role in resistance to drought stress. In tomatoes, drought conditions induce the formation of a class of MAPKs called SIMAPK3, which improves crop drought tolerance by protecting plants from oxidative damage. Its mutants reduce the expression of several genes corresponding to drought stress in the crop, leading to plant wilt, accumulation of antioxidant enzymes, high hydrogen peroxide content, and cell membrane damage. The gene SLNPR1 is related to disease development but not expressed [7], and its knockdown using CRISPR-Cas9 leads to a reduction in the expression of some of the related genes, resulting in reduced drought tolerance in the mutant. The general technical route for gene knockdown is to insert regulatory elements such as promoters or enhancers near the target gene. Traditionally, it is necessary to construct knockout vectors and transfer them into plants through Agrobacterium-mediated transformation, etc. The target gene knockout mutants can be obtained after 1-2 generations, but they can only reduce the expression level of the gene in the crop.

CRISPR-Cas9 technology provides more possibilities for the improvement of drought resistance in crops and is currently used more in wheat and rice.

3.2. Salinization

Due to mismanagement of water resources, the land is facing severe salinization, higher salt ion concentration in the soil will limit the water and nutrient uptake, many crops cannot survive in high salt environment and can cause changes in various cells of the crop, so improving salt tolerance is an important direction to improve crop varieties.

The OsRR22 gene is a negatively regulated gene responsible for encoding a 696 amino acid b-type response-regulated protein transcription factor, and studies have shown that the deletion of OsRR22 significantly improves salt tolerance in rice [2]. Therefore, by precisely editing it with the help of CRISPR-Cas9, rice plants with mutations in the OsRR22 gene were bred, and the mutants had stronger stomatal closure and higher ABA content, realizing that they showed higher survival and improved salt tolerance under salt stress. Currently, there are rice varieties that have been developed using this technology, such as Yanfeng 47. In addition, CRISPR can also be borrowed to study other salt stress response factors, such as MIR52 in rice, RBOHD in pumpkin, SAUR4 in Arabidopsis thaliana, the ACQOS gene cluster in Arabidopsis thaliana, HAG1 in hexaploid wheat, and HVP10 in barley, etc. [8].

Further research on salt stress is yet to be done, and despite the progress made in salt stress biology in recent years, there are still relatively few studies on the phenomenon of salt stress perception.

3.3. Heat and cold stress

In the face of high temperature stress, in addition to resisting it by performing photosynthesis, adjustment of water metabolism, osmotic adjustment, and development of the root system, plants can also reduce the damage by synthesizing heat-stimulated proteins (HSPs), which help other proteins to fold in the correct way and prevent protein denaturation at high temperatures or by accumulating antioxidant substances to scavenge the generated reactive oxygen species (ROS) and increase the plant detoxification capacity. The AFP1 gene regulates HSPs or other genes related to heat tolerance in rice and is involved in high temperature stress response. This gene can be knocked out with the help of CRISPR-Cas9 technology to improve heat tolerance along with other agronomic traits, with a decrease in plant height and fruit set, an increase in effective tillers, a significant increase in panicle length, and variations in yield per plant between 4.06% and 11.75%. Other researchers have improved plant heat tolerance by creating AFP1 mutants. In lettuce, NCED4 gene [9] is one of the key genes for the synthesis of ABA, which affects the cold tolerance of the crop by influencing the synthesis of

ABA. Knockdown of the gene NCED4 using CRISPR technology can enable seeds to germinate under high temperature stress conditions.

In the face of low-temperature stress, plants usually change the composition of cell membrane lipoic acid and accumulate protective substances such as carotenoids and maltose, and low temperatures also prompt the production of antioxidant enzymes to scavenge oxygen radicals or regulate the expression of a series of genes responsive to low-temperature stress (CORs), etc. OsMYB30, located on chromosome 2 of rice, is a negative regulator of cold tolerance in rice, and it interacts with OsJAZ29 affects β¬-amylase gene expression, which in turn affects starch degradation and maltose accumulation, and maltose can act as a compatible solute to improve cold tolerance. It has been shown that plants with simultaneous mutations in three genes, OsPIN5b, GS3 and OsMYB30, based on CRISPR/Cas9 genome editing have improved cold tolerance [9].

4. Conclusion

In this paper, we mainly analyzed the principles and design ideas of CRISPR-Cas9 tools and their applications in helping crops resist some abiotic stresses. CRISPR-Cas9-mediated gene editing technology has become a popular trend, which can directly study the relationship between genotypes and phenotypes, and is now widely used to improve crop traits and to enhance drought, salt tolerance, and extreme temperature resistance of crops through gene knockout and knock-in methods. and temperature extremes through gene knockouts and knock-ins. This technology provides a powerful tool for creating germplasm for beneficial traits, developing new crops, alleviating the food crisis, and creating more sustainable agricultural systems.

A small number of studies have referred to multiple gene editing technology, taking advantage of the CRISPR technology's ability to achieve multiple genome editing by editing multiple sgRNA targets in the genome, which has proved to be a major challenge in future research to make multiple gene editing more widely available with the help of the CRISPR technology. CRISPR also has limitations in terms of off-targeting, which may cause the creation of unexpected mutations and the development of new genes. CRISPR also has the limitation of off-targeting, which may cause unexpected mutations; the current CRISPR-Cas9 is relatively large, which makes it difficult to be loaded into viral vectors, so it needs to be introduced into smaller sizes; and the need to get the approval of the relevant authorities for the crops that have been improved by gene editing is also a problem worth thinking about. This paper discusses only some of the applications of CRISPR-Cas9 technology in abiotic stress resistance and is not comprehensive.

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