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Application of CRISPR/Cas system in rice

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Abstract. Rice, as an essential food in human life, is in increasing demand for food in today's society with such a large population. CRISPR, as the most popular gene editing system at present, has the characteristics of precision, low cost, and simple operation, so it is able to modify the genes of rice to a large extent in order to control its traits and cultivate excellent varieties. In order to better address the demand for food in society, this article conducts gene editing on rice from the perspective of using the CRISPR/Cas system. It provides a brief introduction to the relevant genes and control methods for controlling rice yield in current scientific research achievements, as well as a specific explanation of the relevant genes and control methods for stress resistance such as salt tolerance, cold resistance, and disease resistance. At the same time, in addition to the summary of the current research status, suggestions and prospects are also put forward for the shortcomings of the CRISPR/Cas system in the application of rice and other plants so far.

Keywords: CRISPR Cas9, rice, resistance, gene editing.

1. Introduction

Plants play an important role in the nutritional health and proper growth of humans and animals. With the growth of the population and the increase in the number of living organisms in modern society, the demand for agricultural crops is increasing. The world's population is facing problems such as increasing demand for food. For crops, there are also problems such as insufficient farmland and lack of crop quality. According to the "China Agricultural Products Industry Competition Pattern and Development Trend Forecast Report, 2022-2026" by the China Industry Research Library, the total import and export of agricultural products in China reached US$230.07 billion in 2019, up 5.5% year-on-year, while the total import and export in the first half of 2020 reached US$115.9 billion, up 7.5% year-on-year, a much faster pace than before. This shows that our demand for crops has increased significantly over time, and this means we need to grow the yield and quality of our crops better.

For the Chinese people, rice is the most basic crop and improving crop yield, quality, and stress resistance is the primary problem that breeders need to solve. Nowadays, although traditional breeding based on recombination has achieved certain results, the problems of long-time consumption, high cost, and low efficiency are still difficult to overcome, making it very inconvenient to use in practice. In recent years, the emergence of gene editing technology has broken the limitations of traditional breeding

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and ushered in a new era. Currently, commonly used editing methods include TALENs, ZFNs, and CRISPR [1-3]. CRISPR is the most advanced gene editing tool for plant biologists, with the main tools applied to plant editing, including Cas9, Cas12a, and Cas12b. The Cas9 nucleic acid endonuclease system is a simple, efficient and selective targeted mutagenesis tool for RNA-directed genome editing [4]. The CRISPR/Cas9 genome editing uses single-guide RNA (sgRNA) designed to recognize a 3base pair protospacer adjacent motif (PAM) in the target DNA. But its application is limited due to its primary recognition of the 5'-NGG-3' PAM site. In contrast, the Cas12a system has the following advantages over the Cas9 system: Firstly, the molecular weight of Cas12a protein is smaller than that of Cas9, facilitating molecular processing; Secondly, Cas12a can catalyze crRNA maturation independently without the assistance of tracrRNA sequence; Thirdly, it mainly recognizes the 5'-TTTV-3' PAM as the site, which is more advantageous in T-rich region editing; Forth, Cas12a's cleavage produces sticky ends, which is more efficient and accurate for DNA recognition and integration. CRISPR/Cas12a extends the scope of Cas9 gene editing and has become another important tool for plant gene editing with its advantages of simple assembly, lower off-target efficiency and so on [5].

In the following sections, the fundamentals of the CRISPR/Cas system and its specific applications will be introduced.

2. CRISPR/Cas

CRISPR/Cas system is the defense system of Prokaryote, which is ubiquitous in bacteria and archaea. Through this system, bacteria and archaea can protect themselves from the influence of exogenous DNA or RNA., The CRISPR/Cas system can be divided into types I, II, and III. Among them, there is only one Cas protein in the type II system, namely Cas9 protein. Its Nuclease cutting functional domain is composed of Ruv C and HNH domains, which can cut target DNA double strands respectively.

A CRISPR-Cas system consists of CRISPR loci and CRISPR-related proteins. The first described and most commonly used type II CRISPR-Cas9 system from Streptococcus pyogenes (SPCas9) is composed of a gene encoding Cas9 protein and sgRNA (composed of two RNA molecules, crRNA and tracrRNA). Cas9 protein contains two domains: RuvC and HNH, which have DNA Endonuclease activity. DSB is induced by the recognition of the original spacer sequence adjacent sequence (PAM) by sgRNA and base complementary pairing, The DSB created by Cas9 can be repaired through HDR or NHEJ afterwards.

So far, the application level of the CRISPER/Cas9 system is relatively concentrated, and it is now possible to achieve targeted editing and integration of plant genes through the use of CRISPER/Cas9. Zhang et al. edited 11 target genes in different subspecies of rice, and the mutation efficiency in T0 generation plants reached 66.7%, with 6 target loci being homozygous genotypes [6]. However, Cas12 has made significant breakthroughs in RNA and pathogen detection due to its cis gene editing function and non-specific trans-cleavage activity [7].

The CRISPR/cas9 system has evolved rapidly since its introduction in 2013, and it has important uses in fighting diseases, studying gene function, disease modeling, etc. The advent of CRISPR/Cas12a has compensated for the limitations of the recognition of PAM in cas9, editing efficiency and off-target effects. Not only extending the gene editing scope of the CRISPR system, but also improving the capability for precise gene editing, providing new ideas for the use of the CRISPR/Cas system for resistance modification in plants.

3. Applications

Studying rice’s structure and genetic function is of great significance and practical value. Traditional gene editing techniques such as TALEN and ZFN have limited forms of action, take a long time, and are difficult to obtain the desired traits. The development and utilization of the CRISPR-Cas9 system has broken this limitation. Due to its advantages of high editing efficiency, simple operation, multiple recognition sites, strong scalability, and wide universality, the research process of the rice genome has made remarkable progress, providing a lot of help in improving rice traits, increasing yield, and ensuring safety.

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3.1. Increase production

Growth duration, grains per hole, plant height, grains per panicle, seed setting rate, thousand grain weight, effective tillering number, and other features all have an impact on rice output. Grain weight, the number of grains per spike, and the effective tiller number are some of the qualities that are strongly associated to one another. Numerous genes have already been identified as being linked to these yield traits.

The genes that control rice tillering include MOC1 (Monoculm 1), LAZY1 (Lazaro-like protein 1), and OsTB1 (Teosinte Branched1). Miao et al. designed sgRNA and dual crRNA through the CRISPR-Cas system to process rice cells and obtained a functional loss mutant of the LA1 mutant LAZY1 gene through Agrobacterium-mediated plant transformation. The sub represents the light green leaf phenotype Chlorophyll b (Chl b) caused by the synthesis defect. At the early development stage, the la1 mutant and the function deletion mutant of LAZY1 gene can be observed as the undeclared tiller diffusion phenotype [8]. Yuan et al. used Japanese rice as a sample and constructed corresponding CRISPR-Cas9 vectors based on the three target sites of DDRC1, DDRC2, and DDRC3 on the CDS sequence of the D3 gene. The offspring were transformed by Agrobacterium tumefaciens, and the homozygous mutant plants at the target site DDRC3 in the offspring had more tillers compared to wild Japanese rice [9].

Genes that regulate rice grain weight, width, and length include GW2 (Grain Width 2), GS3 (G protein gamma subunit 3), DEP1 (DENSE PANICLE 1), and CLG1 (Chang Li Geng 1). Meng et al. used the CRISPR-Cas9 system to edit and control the grain length gene GS3, obtained transgenic plant T0, and cultured it in offspring. Then, sequencing analysis was performed on offspring T1 plants, and it was found that some of them underwent single base insertion mutations and some underwent base deletion mutations, And the grain length and grain size were significantly increased compared to the wild type [10]. Shen et al. constructed a co-knockout vector pC1300-2 using the CRISPR/Cas9 system, with the control grain type gene GS3 and the control grain number per spike gene Gn1a as editing objects × 35S: Cas9-gGS3-gGn1a, mutant gs3 and gs3gn1a have longer grain length, increased thousand grain weight and significantly increased grains per spike compared to the wild type. The thousand grain weight of the gs3 mutant increased by 2%~21% and the Gs3gn1a mutant increased 1000 grain weight by 7%~16% [11].

The genes that control the number of grains per spike include IPA1 (Ideal plant architecture1), Ghd7 (Gains Height Date-7), OsMADS17 (SEPALLATA like MADS box 17), and Gn1a (G rain number 1a). The Gn1a, DEP1 (Os09g0441900), GS3, and IPA1 genes of Zhonghua 11 have been described and validated as regulatory factors for grain number, spike structure, grain size, and plant structure. Li et al. altered these genes using the CRISPR-Cas9 system. The T2 generation of the Gn1a, DEP1, and GS3 mutants, respectively, displayed larger grains, a more compact and erect plant type, and an increase in grain number. The DEP1 and GS3 mutant plants also have semi-dwarf and long-awn grain characteristics respectively. Among them, the IPA1 mutant exhibits two distinct phenotypes due to different target regions of OsmiR156 in vivo: producing fewer or more tillers [12].

According to the aforementioned findings, CRISPR-Cas9 can facilitate the analysis of intricate gene regulatory networks with the same genetic background by altering regulatory factors for a variety of significant traits. It can also offer potential plant breeding techniques for enhancing yield traits of current planting varieties, which will speed up rice breeding.

3.2. Resistance

The stress resistance of rice usually includes multiple aspects such as cold resistance, drought resistance, salt tolerance, disease and pest resistance. These traits are not only directly related to the production capacity of rice, but also have important connections with its quality and taste. Therefore, studying the improvement of stress resistance traits in rice can help better understand the growth and development process of rice, and provide new ideas and methods for improving rice yield and quality.

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3.2.1. Salt tolerance. Soil salinization is one of the main stresses that restrict crop growth. Salt stress has an impact on the nutrient metabolism of cells at different stages of crop development, and is one of the main reasons for yield decline. Therefore, improving the salt tolerance of rice is a committed step to improve rice productivity. At present, CRISPR-Cas has been widely used in the research of salt stress-related genes in rice due to its powerful advantages, such as OsDST, OsSOS1, OsHKT, OsWRKY13, OsSNAC1, OsMYB, OsNAC, etc. Yang et al. found through research that mutations in the OsEIL1 and OsEIL2 genes lead to a decrease in the expression of OsHKT2, reducing the absorption of sodium ions by roots, and thereby enhancing salt tolerance in rice. Mo et al. used the japonica rice variety Dongnong 427 as the experimental material and used the CRISPR-GE online toolkit to design a knockout site near the 5 'segment of OsEIL1 and OsEIL2 genes. Then, they used the sgRNA of the CRISPR-Cas system to simultaneously knock out two genes. Afterwards, they used Agrobacterium tumefaciens transformation for offspring culture and found that the survival rate of seedlings under salt stress was significantly higher than that of the wild-type, which was about 75.0%. This successfully modified the salt tolerance of the rice variety Dongnong 427 [13].

3.2.2. Cold resistance. As a cold-sensitive crop, rice is particularly affected by cold damage, and the yield of rice affected by cold damage can be reduced by more than 10%. Especially during the seedling stage, rice under cold stress may experience slow growth, withered leaves, and stunted plants. Therefore, exploring and modifying cold tolerance genes to cope with low temperatures and cold damage is particularly important for ensuring rice yield. Wang et al. studied a known rice cold tolerance gene OsRab11C1, encoded and synthesized gRNA based on this gene, and constructed a CRISPR-Cas9 vector. The gene was transformed into Japanese rice through Agrobacterium tumefaciens. Under three consecutive low-temperature treatment experiments, the seedling length and root length of rice offspring were significantly improved due to the wild type, and the visible cold tolerance performance was significantly improved. In addition, the gene was derived from rice, so the improved variety obtained reduced concerns about food safety. Wang's research reveals a way to regulate rice cold tolerance. Further research can explain the molecular mechanism of regulating rice cold tolerance and help broaden the scope of rice gene editing research [14].

3.2.3. Disease resistance. Rice blast is the most destructive disease in rice production, caused by the filamentous ascomycete fungus Magnaporthe oryzae. It can damage almost all parts of the rice growth process, often causing serious impacts on global rice production. Enhancing host resistance has now been found to be the most economical and efficient way to control rice blasts through studies on several elements of the disease. After years of research and development, CRISPR Cas, also known as a sequence-specific nuclease (SSN), has been recognized as a powerful tool for improving genome editing. Wang et al. designed CRISPR-Cas922 (C-ERF922), which targets the OsERF9 gene in rice, and transformed the binary vectors expressing Cas9/sgRNA (pC-ERF922, pC-ERF922S1S2 and pC-ERF922S1S2S3) into rice protoplasts to obtain rice variety Kuiku131. They selected 21 ERF922-S2 Mutant plants from 50 positive transgenic (T0) plants for analysis, and found that all C-ERF1 induced allelic mutations can be passed on to offspring, Subsequently, further detection and analysis were conducted on villages and mutant T2 with different types of allele mutations. The results showed that the number of lesions formed by pathogen infection in the mutant lines during the seedling and tillering stages was significantly reduced, and the susceptible area was reduced by nearly 50%, improving their resistance to rice blast disease [15].

Therefore, using CRISPR/Cas9 technology to target key functional genes has the potential to become a promising biotechnology strategy for promoting rice breeding.

4. Conclusion

As the most popular gene editing technology at present, the CRISPR/Cas system has demonstrated significant potential for the excellent development of rice through its research on yield and resistance. However, there are a number of challenges associated with the use of the CRISPR/Cas system in plant

research., including Firstly, the widespread availability of the anti-CRISPR protein and the need for in-depth analysis of the resistance produced by the CRISPR/Cas protein to see the effects on cells; Secondly, the need to introduce the CRISPR protein gene and the vector for accessory integration when editing the CRISPR/Cas system. The expression of the vector in the plant needs further study in plant growth and development; Thirdly, in terms of editing plant resistance to viruses, the impact of gRNA on virus evolution needs to be considered for viruses that are prone to mutate themselves to escape host monitoring.

In order to solve these problems, a feasible approach is to search for a protein which can weaken the transcription of repressor proteins. In addition to this, it is possible to find or synthesize a protein that prevents the anti-CRISPR protein from binding to CRISPR, reduces its viability, competes with the anti-CRISPR protein for binding sites, or inactivates the anti-CRISPR protein; or develops a chemical that acts directly on the anti-CRISPR protein by exogenously introducing a sequence of faulty bases and expressing the anti-CRISPR protein with a faulty amino acid sequence. Inactivation by altering the spatial structure. In this way, the anti-CRISPR protein cannot function and CRISPR can work properly.

Authors contribution

All the authors contributed equally and their names were listed in alphabetical order.

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