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Applications of CRISPR/Cas9 system in improving plant genomes

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Abstract. At present, the clustered regularly interspaced short palindromic repeats/CRISPR-associated protein-9 (CRISPR/Cas9) system is the most concerned gene editing tool in the world. It was first identified in Escherichia coli in 1987 by Japanese researchers Y Ishino et al. And it has rapidly developed into a mature and advanced genomes engineering tool technology. Due to its easy operation, inexpensive and other characteristics, it has replaced other programmable nucleases such as zinc finger nucleases (ZFNs) and transcription activator-like effector nucleases (TALENs), and has become the most widely used method to edit genomes in many fields such as agricultural production and cancer treatment. Editing plant genomes with CRISPR technology can effectively enhance the resistance of viruses and pests in plants. This review will list some applications of CRISPR/Cas9 system in improving plant genomes. Although it has been widely used, the off-target effects are the major challenge to be solved. This review will also outline current difficulties and offer a possible direction for CRISPR/Cas9 system development in the future.

Keywords: CRISPR/Cas9 System, Engineering Tool Technology, Zinc Finger Nucleases, Transcription Activator-Like Effector Nucleases, Off-Target Effects.

1. Introduction

Agricultural production is a necessary guarantee for human development. With the world's population growing, the demand for food will be increasing. According to the data published by the United Nations, the global population has reached 8,045,311,447 in 2023, an increase of 0.88% over 2022. In addition, the world's population is expected to reach 8.5 billion by 2030 ,and it is probably to continue to grow after that. In this case, how to enhance crop yield and safety has become a global topic. At present, agricultural production directly affects the quality of human life, and how to improve crop yield is a great challenge for human beings.

Gene editing technology is a technology to change the target gene sequence, achieve site-specific mutation, insertion or knockout, and it can modify the plant genome to achieve the purpose of increasing yield. With the continuous progress of gene editing technology, more and more gene editing tools have been applied to agricultural production such as utilizing zinc finger nucleases (ZFNs) to modify the maize mutations in IPK1 to create low phytate lines [1]. Zinc finger proteins (ZFPs) and the type IIs restriction endonuclease FokI's non-specific cleavage domain make up ZFNs [1]. ZFPs is a typical eukaryotic gene transcription regulator that is crucial for regulating gene expression, cell differentiation, embryonic development, and improving plant stress resistance [2]. TALENs is another

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flexible and efficient targeted editing technology after ZFNs. TALENs is similar to ZFNs in structure and consists of two parts: transcription activator-like effectors (TALEs) protein and FokI nuclease. Each of the 33 repeats in the TALE DNA-binding domain has the potential to differ by two amino acids or two acids, which affects which nucleotide it will bind [2]. Combining 12 to 31 of these repeats will allow a TALEN to be designed to attach to a particular DNA sequence. A DNA double strand break can only be made when two TALENs dimerize. TALE protein is a particular DNA-binding protein as well as a transcriptional activator-like effector released by Xanthomonas spp [3]. It can specifically identify and bind host target gene sequences in plant cells, controlling the expression of the host gene. For enhancing the disease resistance of rice, it is possible to utilize TALENs technology to modify the promoter sequence of the Os11N3 gene, which causes the TAL effectors AvrXa7 and PthXo3 to lose recognition of the Os11N3 promoter sequence [3].

The above methods to target edit plant genomes are the two common editing tools at present. Alongside CRISPR/Cas9 system, they are also prominent tools in the field of genome editing. The cell can repair the double stranded DNA in these three systems using the NHEJ and HDR repair pathways [3]. To complete gene knockout, selection must be used to identify cells containing frameshift mutations. However, ZFNs and TALENs editing techniques are more expensive and difficult to operate than CRISPR/Cas9 system [4]. It is the reason why CRISPR/Cas9 system is the most widely used technology for modifications of plant genomes.

History:

CRISPR, which exist in bacteria and most archaea, is a product of prokaryotes that evolved over time to fight viruses. The CRISPR/Cas9 system evolves from the type II CRISPR-Cas system [5]. In 1987, the first article on CRISPR was reported by Japanese researchers Y et al., and referred to it as a short regularly repeated sequence detected in Escherichia coli [4,5]. Then in 2000, it was detected in bacteria and most archaea. It was renamed CRISPR in 2002 [6]. CRISPR/Cas was first used in biotechnology in 2007 and mature CRISPR RNA was proved to be able to resist foreign viruses or plasmids in the next year [6]. Recent years have seen an increase in the publication of research articles on CRISPR as a result of the quick development of technology, which has had a significant impact on a number of various areas.

2. Structure and Principle

The Cas9 protein and single-stranded guide RNA (sgRNA) are the two main elements of the CRISPR/Cas9 system; the Cas9 protein serves as a tool for cutting double-stranded DNA and the sgRNA serves as a guide. Cas9 protein can cut on several target sites in the sgRNA guide through the concept of base complementary pairing to achieve double-stranded DNA breaks [7].

The immunological response of the CRISPR/Cas system is separated into three phases and is modeled after the adaptive defense of bacteria and archaea. The first stage is the acquisition stage, where phage and exogenous DNA enter the bacteria and are recognized and incorporated into the CRISPR array to generate immune memory [7, 8]. The second stage involves the expression of the Cas protein and the transcription of the CRISPR array to create crRNA precursor (Pre-CRISPR RNA), which is later transformed into mature CRISPR RNA by trans-activation and catalyzation by ribonuclease III (RNaseIII) [8]. Finally in the interference stage, when the phage invades again, the CRISPR/Cas system cuts the genome of the phage and the plasmid genome by Cos protein under the guidance of CRISPR RNA to form DSBs [8].The specific process can be divided into the following three steps.

2.1. Acquisition of highly variable spacer regions for CRISPR

The proteins encoded by Cas1 and Cas2 recognize exogenous DNA as it enters the cell and look for the PAM region of the DNA [6]. The DNA sequence close to the PAM region will then be utilized by CRISPR as a potential protospacer. The Cas1 and Cas2 protein complex shears the chosen interval sequence after recognition [6]. The original spacer sequence is simultaneously inserted by a component of the enzyme into the leader region of the CRISPR sequence's downstream region. Normally, the broken DNA is self-repaired with a highly effective non-homologous end-junction (NHEJ) method,

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which closes the double-stranded gap that has been cut. After this stage, the genome's CRISPR sequence gains a new interval sequence [6-8].

2.2. Expression of the CRIPSR locus

The leading region of the CRISPR sequence regulates the transcription of the sequence to produce pre-CRISPR RNA, at which point tracrRNA is transcribed to complement the pre-CRISPR RNA sequence [9]. Through base complementary pairing, the pre-CRISPR RNA joins forces with tracrRNA to create a double-stranded RNA, which is then assembled with Cas9-encoded proteins [9]. Depending on the kind of exogenous DNA, the complex will choose a distinct internal spacer region sequence of RNAs and shear them with the help of RNase III to create a short CRISPR RNA. (containing a single type of internal spacer region sequence of RNA and a partial repeat region).

2.3. The role of CRISPR/Cas9 system

The complete exogenous DNA sequence will be recognized by the complex of CRISPR RNA, Cas9, and tracrRNA, as well as the protospacer that is complementary to the crRNA. The DNA double strand is then unraveled to generate an R-loop as the complex localizes to the PAM/protospacer area. While the complementary strand is still free, the crRNA is joined to it in the meantime. In order to create the flat-end product, the Cas9 protein creates a precise flat-end cleavage site three nucleotides upstream of PAM [10]. While the RuvC domain of the Cas9 enzyme cuts the other non-complementary DNA strand, the HNH domain of Cas9 cuts the complementary DNA strand to the crRNA [10]. Eventually, Cas9 results in a DNA double-strand break (DSB), which inhibits the production of foreign DNA.

3. Applications

CRISPR/Cas9 system can achieve fixed-point knockout, insertion and replacement of genes etc. Thus, it can quickly and efficiently achieve the improvement of plant traits, and has important significance for the research and application of plant gene function. At present, the use of CRISPR/Cas9 system-mediated plant genome editing technology primarily entails plant functional genomics research, superior crop variety breeding, and targeted use of CRISPR/Cas9 system to convert crop varieties, enhance plant disease resistance, boost yield, and so forth.

3.1. Rice

Miao et al. improved the Cas9 gene's codon sequence in 2013 to obtain extremely effective expression in rice. The targeted deletion of the OsFWL4 gene can also enhance the number of tillers and rice yield, and the optimal systematic deletion of the OsLAZY1 gene, which regulates tiller angle, can drastically alter rice plant type [11]. The research findings showed the viability of utilizing CRISPR/Cas as molecular scissors to create DSB at specified places of plant genome to achieve targeted genome modification in monocotyledon plants in the same year that Feng et al. chose the ROC5, SPP, and YSA genes of rice for editing [12]. In 2016, a team of researchers used CRISPR/Cas9 technology to edit the rice blast infection effect gene OsEFR922 and produce progeny plants with only the target gene altered without undergoing transgenic transformation. This significantly reduced the spore damage that developed after pathogen infection compared to wild-type plants [13]. In 2017, Using CRISPR/Cas9 technology, Tang et al. shut down the metal transporter gene OsNramp5 in rice, creating a new indica strain with reduced cadmium (Cd) accumulation without lowering yield. This offers an efficient way to create indica rice that is protected from Cd contamination [13]. Zhang et al. were able to transform japonica rice into glutinous rice by editing the Waxy gene with the CRISPR/Cas9 system, which encodes granulose-binding starch synthase I. By editing the gene's promoter, they were able to control the gene's expression and achieve their goal of regulating amylopectin content in 2018 [13]. In order to create a new strain of rice in 2020 [14], CRISPR/Cas9 was used by Ashokkumar et al. to create a novel allele of OsBADH2. They then inserted it into superior non-aromatic rice cultivars. Through targeted mutagenic transformation, the CRISPR/Cas9 system and other gene editing technologies have created a new technique to speed rice quality

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improvement. Researchers completed site-specific replacement of endogenous OsEPSPS and OsALS genes in rice in 2021 using the CRISPR/Cas9 system generating NHEJ repair to make site-specific replacement and site-specific insertion of genes [13].

3.2. Wheat

In 2019, Okada et al. developed hexaploid wheat non-transgenic materials with totally homozygous male sterility features by deleting the wheat Ms1 male sterility gene with the aid of CRISPR/Cas9 gene editing technologies. Through this technique, T1, T2, and T3 generations of wheat could receive the recessive ms1 allele. To create wheat male sterility mutants, Li et al. modified three homologous TaNP1 alleles using CRISPR/CAS9 technology [15].

3.3. Corn

The size of the pistil meristem and the length of the corn ear decreased after Yun et al. employed the CRISPR technique to remove YIGE1 [16]. By realizing the recombination of chromosome regions with a significant number of genetic variants, Dupont employed CRISPR/Cas9 technology to realize the 75.5 Mb interarm inversion of the high-quality maize inbred line PH1V5T chromosome [17]. This created a new gene resource for maize breeding.

3.4. Soybean

In 2015, Jacobs et al. published the first study on the site-directed mutagenesis of soybean utilizing CRISPR/CAS9 technology. This work provided a solid framework for modifying soybean genomes. Since then, the CRISPR technique has been extensively utilized to verify gene function and enhance soybean varieties. In order to reduce soybean plant height and shorten internode length, CRISPR/Cas9 was utilized by Qun et al. to cause a site-specific mutation in the GmLHY gene. This study serves as a foundation for the analysis of the potential mechanism of the network that regulates soybean plant height [16]. Shi et al. edited the GmBADH1 gene CRISPR/Cas9 technology was used to transfer the vector into the JACK receptor using Agrobacillus-mediated soybean genetic transformation technology, and discovered three different stable genetic mutations without T-DNA insertion [16]. After the GmBADH1 gene was deleted at the seedling stage, the salt tolerance of soybean significantly decreased, but there was no effect at the seedling stage, indicating that this gene mainly played a positive regulatory role in the salt tolerance of soybean at the seedling stage. This resulted in a foundation for people to further explore the salt tolerance regulatory genes at various stages of soybean [16].

Table 1. Other applications of CRISPR/Cas9 system in plants.

Plant	Target Gene(s)	Function
Rice	OsSPL16/qGW8	The yield of grains greatly increased [13]
	Bsr-d1 Pi21 and ERF92	Improved the resistance to Rice blast and bacterial blight [13]
	OsSBEIIb	Significantly more resistant starch was present [17]
	OsVQ25	Improved the broad-spectrum resistance to Rice blast and bacterial blight [17]
	OsALS	Confer resistance to sulfonylurea herbicides [17]
	PIN5b GS3 and MYB30	Obtained the high-yield and hardy rice [18]
Wheat	TaSBEIIa	The content of resistant starch increased significantly [13]
Corn	ZmWX and ZmSH2	Created a new sweet glutinous corn [17]

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Table 1. (continued).

Cotton	GhFAD2	The content of cottonseed oleic acid increased, while the content of linoleic acid decreased [13]
Citrus	LOB1	Enhance the resistance of citrus canker [18]
Tomato	SlIAA9	Obtain parthenocarpy tomato [18]

All in all, the current CRISPR/Cas9 system has become a cutting-edge biotechnology tool for gene function research and crop trait improvement, playing a greater role in improving crop growth, development, cultivation of high quality, high yield and tolerance to biological and abiotic stresses, and its application in production practice is also increasingly wide.

4. Prospect

The advancement of CRISPR/Cas9 technology has created new avenues for plant genetic engineering. CRISPR/Cas9 performs as expected as a site-directed mutagenesis tool for genome editing. High efficiency, high specificity, low price and easy operation are beyond the reach of traditional mutagenesis methods. Off-target effect continues to be the main barrier to the CRISPR/Cas9 system's further and broad adoption at this time. Therefore, additional study is required to enhance target specificity in order to prevent or reduce the off-target effect.

Generally, the CRISPR/Cas9 system's Cas9 endonuclease and sgRNA components are the principal subjects of research on how to reduce off-target effects.. For example, the ideal sgRNA sequence is developed to reduce the possibility of missing the target. On the other hand, it is also possible to consider improving precision gene editing with the CRISPR/Cas9 system. The second issue is the existing CRISPR/Cas9 system's comparatively poor editing efficiency. Therefore, the researchers hope to improve the above problems by the following methods. (1) First, by concentrating on DNA repair pathways, editing efficiency can be increased. The frequency of homologous recombination (HR) pathway is very different in different species and cell types. HR path mainly occurred in S and G2 stage. Non-homologous end-joining (NHEJ) pathway mainly occurs in G1, S, and G2 phases, and the selected repair pathways differ from cell cycle to cell cycle. Genome editing can be done by synchronizing the cell cycle, which can improve editing efficiency. For example, Baltas et al. used tobacco as the research material and expressed SSNs and DNA templates using Geminivirus vector [19]. Compared with T-DNA vectors, the efficiency of HR pathway has been greatly improved. Moreover, to increase the effectiveness of editing, the CRISPR/Cas9 system can also be improved, such as the optimization of core components.

Thirdly, there is the issue of government regulation of gene-edited crops. With the development of CRISPR/Cas9 technology, more and more scholars are using it to improve varieties, which can reduce the time required for years or even decades to a few months. More importantly, CRISPR/Cas9 technology is able to obtain desirable characteristics and pass them on to future generations, but what kind of results the resulting gene-edited crops will bring to the world is unknown, and people are very concerned about it. At present, governments around the world have two main regulatory attitudes towards genetically modified organisms (GMOs) : (1) Process-based regulation represented by the European Union (EU), and gene recombination technology is considered to be potentially dangerous. No matter what genes or organisms are obtained through this technology, as long as external DNA is transferred, even if there is no foreign DNA in the body, safety assessment and monitoring are needed. (2) Product-based regulation, represented by the United States, believes that there is no essential difference between GMOs and Non-GMOs, and it is the products produced through biotechnology that need to be regulated, rather than the technology itself. In contrast, process regulation is stricter than product regulation. U.S. agricultural regulators believe that gene-edited crops (GECs) are mutations created by activating DNA repair mechanisms in cells, similar to natural mutations, physiochemically induced mutations, and are not GMOs. Due to the influence of various factors, the EU is very cautious towards GMOs and very conservative towards NBTs such as transgenic and gene editing, resulting in its related

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industrial pattern and product development falling behind that of the United States and other countries. However, at present, there is no clear regulation on the regulatory standards of GECs, and there is no relevant report on its regulatory attitude. Although genome editing technology has only existed for more than a decade, its impact on agriculture and medicine is very significant. The global industrial pattern based on genome editing technology is gradually taking shape, thus, it is very important to formulate reasonable regulations for the research progress of genome editing technology to correctly guide the development and application of genome editing technology.

Additionally, current CRISPR/Cas9-based alterations to the plant genome usually entail the mutation of particular loci and the deletion of small segments between two target sites, which typically results in the target gene's loss of function. The technical difficulties of carrying out exact replacement, insertion, and deletion operations on certain segments of the plant genome and creating heritable plants, on the other hand, have not yet been fully overcome. Therefore, the direction of future efforts will surely be toward the development of this higher level of genome modifying technology.

5. Conclusion

The development of the CRISPR/Cas9 system will revolutionize basic research and applications in plants. However, concerns have also arisen about the potential misuse of this technology. However, compared to medical and animal research, plant genome modification involves basic and applied research without ethical issues, and therefore the CRISPR/Cas9 system has more freedom to be used in plants. Biologically speaking, the nature of gene targeting mutation based on CRISPR/Cas9 and other genome editing technologies is substantially equivalent to that of traditional random gene mutation (natural or artificial mutation), but genome editing targeting mutation is more targeted and efficient. Moreover, mutant strains free of transgenic elements can be obtained through genetic segregation of the progeny, and therefore genome editing techniques should have a higher degree of safety than traditional mutation breeding methods.

In summary, the emergence of CRISPR/Cas9 system is expected to help us better understand the functions, mechanisms and interactions of plant genes, and will also provide strong support for the realization of the precise regulation of plant quality. In addition, the application of gene editing technology is also expected to help the agricultural field to realize efficient and non-polluting production methods, thus further improving the production capacity and quality of food.

References

[1] Joseph F Petolino 2015 Genome editing in plants via designed zinc finger nucleases In Vitro Cell Dev Biol Plant. 51(1):1-8

[2] J. Keith Joung & Jeffry D. Sander 2013 TALENs: a widely applicable technology for targeted genome editing Nature Reviews Molecular Cell Biology volume 14, pages 49–55

[3] Liao et al 2016 Effects of γ Ray Irradiation with Different Dosages on Strawberry Seeds and their Genetic Diversity Revealed by ISSR Fenzi Zhiwu Yuzhong (online) (Molecular Plant Breeding), 14(10): 1062-1071

[4] Xuan Liu, Surui Wu, Jiao Xu, Chun Sui and Jianhe Wei 2017 Application of CRISPR/Cas9 in plant biology Acta Pharm Sin B. 2017 May;7(3):292-302

[5] Doudna, J. A., & Charpentier, E 2014 The new frontier of genome engineering with CRISPR-Cas9 Science, 346(6213), 1258096

[6] Hsu, P. D., Lander, E. S., & Zhang, F. 2014 Development and applications of CRISPR-Cas9 for genome engineering Cell, 157(6), 1262-1278

[7] Milan Kumar Samanta, Avishek Dey and Srimonta Gayen 2016 CRISPR/Cas9: an advanced tool for editing plant genomes Transgenic Res. 2016 Oct;25(5):561-73

[8] Khaoula Belhaj, Angela Chaparro-Garcia, Sophien Kamoun, Nicola J Patron and Vladimir Nekrasov 2014 Editing plant genomes with CRISPR/Cas9 Curr Opin Biotechnol. 2015 Apr;32:76-84

DOI: 10.54254/2753-8818/16/20240555

[9] Jake Adolf V. Montecillo, Luan Luong Chu and Hanhong Bae 2020 CRISPR-Cas9 System for Plant Genome Editing:Current Approaches and Emerging Developments Agronomy, 10(7), 1033

[10] Virginia M. G. Borrelli, Vittoria Brambilla, Peter Rogowsky, Adriano Marocco and Alessandra Lanubile 2018 The Enhancement of Plant Disease Resistance Using CRISPR/Cas9 Technology Front. Plant Sci

[11] Qingsong Gao, Gang Li, Hui Sun, Ming Xu, Huanhuan Wang, Jianhui Ji, Di Wang, Caiyong Yuan and Xiangxiang Zhao 2020 Targeted Mutagenesis of the Rice FW 2.2-Like Gene Family Using the CRISPR/Cas9 System Reveals OsFWL4 as a Regulator of Tiller Number and Plant Yield in Rice Int J Mol Sci. 2020 Jan 26;21(3):809

[12] Xu Jifa, Xu Yan, Zhao Jiqiang, et al 2017 CRISPR/Cas9 system and its application in Monocotyledonous plants [J]. Jiangsu Agricultural Sciences, 45 (18) : 21-24

[13] Liu Di, Liu Linlin, Zhen Junbo, Du Haiying, Ouyang Yanfei and Chi Jina 2023 Research progress of CRISPR/Cas9 gene editing technology in plants and its application in cotton Molecular plant breeding, ISSN 1672-416X,CN 46-1068/S

[14] Ashokkumar S., Jaganathan D., Ramanathan V., Rahman H., Palaniswamy R., Kambale R. and Muthurajan R. 2020 Creation of novel alleles of fragrance gene OsBADH2 in rice through CRISPR/Cas9 mediated gene editing." PLoS ONE, 15(8): e0237018

[15] Li J., Wang Z., He G., Ma L. and Deng X.W 2020 CRISPR/Cas9-mediated disruption of TaNP1 genes results in complete male sterility in bread wheat J. Genet. Genomics, 47(5): 263-272

[16] Shi Yuxin, Liu Xinyue, Sun Jianqiang, Li Xiaofei, Guo Xiaoyang, Zhou Ya, Qiu Lijuan 2023 Using CRISPR-CAS9 Modification of GmBADH1 gene modified salt tolerance of soybean [J/OL]. Journal of Crop Science

[17] Jiang J, Sue H, Hong D et al 2023 Research progress of plant biotechnology Plant Physiology, 59 (8): 1436–1462

[18] Long Y, Zeng J, Wang L et al 2023 Advances in CRISPR/Cas9 genome editing technique and its application in medicinal plants Chinese Traditional and Herbal Drugs 2023 May Vol. 54 No. 9

[19] Baltes NJ, Gil-Humanes J, Cermak T, et al. 2014 DNA replicons for plant genome engineering [J]. The Plant Cell, 2014,26(1) 851 -1932