# The application of gene-editing technologies in plants

Yi'e Qin *

College of Life Sciences, Northwest A&F University, Shaanxi, China

* Corresponding Author Email: qinye@nwafu.edu.cn

**Abstract.** In recent years, we have witnessed a spurt of progress in modern molecular biology, thus enabling the perfection of genome sequencing. As a result, research into gene function has gained popularity among scientists. Since gene editing has served as an essential tool for the exploration of plant gene function, the utilization of genome editing technology contributes most to the genetic upgrading and crop breeding through modification on plant genome. Typical cases where CRISPR/Cas system has been applied to model species including Arabidopsis thaliana, Nicotiana tabacum and Oryza sativa to alleviate salinity stress and figure out the gene behind traits relative to their architecture and yield. The modified CRISPR/Cas9 system requires only simple cloning compared with the other two types of gene editing tools and the design of target sequences is easy, the construction of backbone vectors is simple, and the cost is relatively low. The CRISPR/Cas9 system, which was awarded the 2020 Nobel Prize in Chemistry, has transformed the molecular life sciences by enabling researchers to accurately modify the DNA of animals, plants, and microorganisms. This review thoroughly introduced gene editing technologies currently applied in plants, including map cloning, RNAi technology and targeted genome editing technology, containing ZFNs, TALEN, CRISPR/Cas9. The review revealed the difficulties and potential uses of genome editing technology in plants while concentrating on its theory and viability.

**Keywords:** Genome sequencing; gene-editing technology; CRISPR/Cas system.

## 1. Introduction

As modern molecular biology advances by leaps and bounds, the sequencing of plant genomes has become much more robust, and the study of gene function has become a research priority. Since the 20th century, researchers have never stopped exploring the gene editing technology in plants, and in order to better study functional genomics, also called post genome, high-throughput DNA microarray and expression profiling data, functional gene mapping cloning, large-scale mutant libraries, high-efficiency RNAi technology, and targeted genome editing technology have burgeoning, which are important technologies for plant functional genome research. These developing technologies are significant for plant functional genome research, which have greatly accelerated the study of plant gene function and have been applied in many aspects [1, 2].

## 2. Fundamentals of plant gene editing research

### 2.1. Positional cloning

Positive cloning is a gene cloning technique developed due to the establishment of molecular marker mappings for various plants. The principle is listed as below: functional genes in the genome have a relatively stable gene locus, in the use of molecular marker technology on the basis of the fine positioning of the target gene after a series of steps, in order to find a clone containing the target gene, and finally confirmed by the genetic transformation test of the function of the target gene [3, 4].

The map cloning technology mainly consists of four basic technical links: (1) the initial positioning of the target gene; (2) fine positioning; (3) the construction of the physical map of the target gene region and the fine physical map until the identification of a smaller genome fragment containing the target gene; 4) screening of cDNA libraries and through genetic transformation experiments to confirm the function of the target gene obtained and sequence analysis [5].

Since 1992, when the map position cloning technique was first applied to Arabidopsis thaliana to successfully isolate the ABI3 gene and the FAD3 gene, it has been applied to more than dozens of other plants, shortening the experimental period while being widely used especially in resistance genes of pathogens and plant signaling pathways, and accelerating the development of molecular biology of pathology.

### 2.2. RNAi technology

RNA interference (RNAi) first being found in the cryptic rod nematode in the showy, is the eukaryotic organisms in the widespread resistance to viral replication and expression, inhibiting transposon transposition and regulating gene expression based on the monitoring mechanism of the principle of dsRNA molecules caused by sequence-specific degradation caused by the target mRNA, finally resulting in silencing of endogenous target genes [6, 7].

The process is ATP-dependent and the typical RNAi pathway is divided into three phases: initiation, effector, and signal amplification. The core mechanism includes: Dicer-like (DCL) enzymes have processed dsRNAs into 21-25 nt-sized siRNAs (small-interference RNAs). One strand of these siRNAs binds to the RNA-induced silencing complex (RISC) and paves the way for the target mRNA's deterioration. Signal amplification requires RDR enzyme (RNA-depend- entRNA polymerase) to use RNA as template, synthesize dsRNA, which is cleaved by DCL into secondary siRNAs, and enter into a new round of RNAi effect. RNAi techniques, with their high specificity, efficiency, systematics and heritability, are an effective way to obtain mutants in plants and are of great importance in developmental biology research. RNAi techniques are highly specific, efficient, systematic and heritable, and are an efficient way to obtain plant mutants, which is important for developmental biology research. In addition, the systematic nature of RNAi and other characteristics also greatly promote the further exploration of plant pest control, disease resistance, and stress resistance [7, 8].

### 2.3. RNAi technology

RNA interference (RNAi) first being found in the cryptic rod nematode in the showy, is the eukaryotic organisms in the widespread resistance to viral replication and expression, inhibiting transposon transposition and regulating gene expression based on the monitoring mechanism of the principle of dsRNA molecules caused by sequence-specific degradation caused by the target mRNA, finally resulting in silencing of endogenous target genes [6, 7].

### 2.4. Spot Editing Technology

Genome editing uses sequence-specific nucleases to generate DNA double-strand breaks on specific spots in the whole genome, thereby activating the cell's self-repairing mechanism - non-homologous end-joining (NHJ). Cell's self-repairing mechanisms can activate two pathways including NHEJ and HR , thus triggering gene knockout, chromosome recombination, and targeted gene insertion or replacement. ZFNs, TALEN and CRISPR/Cas9 use protein-DNA binding to target specific genomic sites, while the latest CIRISPR/Cas9 system uses simpler complementary nucleotide pairing to bind to genomic targets, which is simpler and more efficient, thus promoting the wide application in plants' genome editing [9].

#### 2.4.1. ZFNs

Zinc finger nuclease (ZFN) is the first artificially modified targeted endonuclease genome editing technology which has met with success to the application in multiple species. It consists of two components: zinc finger protein (ZFP), which specifically recognizes DNA, and FokI endonuclease, which cuts DNA double strands in a non-specific manner. Pairs of ZFPs bind specifically to the sequence of target DNA, and FokI at C-terminus of two ZFPs form a cleavage-active dimer, which generates the DSB, which is then repaired by the cell's own DNA repair pathway, because errors

inevitably occur during the repair process, and these incorrect repairs constitute site-specific genome editing [10].

Zinc finger proteins consist of ZFP motifs with specific DNA recognition capabilities, where each ZFP motif recognizes a triplet of bases such that a tandem ZFP motif can specifically recognize a section of DNA sequence. Typically, the structural domain of zinc finger proteins consists of 3-6 zinc finger protein motifs in tandem, but the ability of tandem zinc finger proteins to specifically recognize base sequences is limited because of the interaction effect between zinc finger protein motifs, i.e., not any zinc finger protein motif in tandem is active [11]. Screening for active tandem zinc finger protein motifs requires large corporate platforms for library building screening, not something that can be implemented by individual labs. This is the most direct reason why ZFNs are not used on a large scale.

### 2.4.2. TALEN

TALEN, short for Transcriptional Activator-Like Effector Nucleases, is another genome editing technique following ZFN.

TALEN is another genome editing technique following ZFN. TALEN consists of two parts: a TALE domain with DNA-specific recognition ability and a FokI endonuclease domain that cleaves DNA sequences (the structure is the same as that of ZFNs). The plant pathogen Xanthomonas campestris determines the binding specificity of TALE. The TALE's DNA recognition binding domain in nature consists of 1.5-33.5 tandem repeats, apart from polymorphisms at positions 12 and 13 which are called RVDs (Repeat Variable Diresidues), each comprising 33 to 35 highly conserved amino acids that are identical. The binding specificity of each TALE repeat are determined by these two amino acids , and there is a correspondence between the RVDs and the recognized bases that allows the design and synthesis of specific tandem TALEs that bind to specific sequences, which are then dimerized by FokIs to produce DSBs, and ultimately mutations. Like ZFNs, TALENs are usually used in pairs, and the length of the recognized genomic DNA sequence can be extended to improve specificity [12]

### 2.4.3. CRISPR/Cas9

Following ZFNs and TALENs, the CRISPR/Cas technique, as a newcomer to genome editing, has enjoyed a wide range of applications because it is easier to construct vectors, less expensive and less technically difficult.

CRISPR, an abbreviated form, refers to clustered regularly interspaced short palindromic repeat sequences, and Cas (CRISPR associated) refers to CRISPR associated proteins.

The CRISPR/Cas system possesses two components: CRISPR sequences and Cas proteins, in which the CRISPR sequences consist of some highly conserved repetitive sequences and spacer sequences interspersed with each other, and Cas proteins are proteases encoded by related genes near the CRISPR sequences, which are nuclease-active, and can cleave the DNA sequences and cause DNA double-strand breaks [13].

The Cas protein is a protease encoded by the relevant gene near the CRISPR sequence, with nuclease activity, which can cut the DNA sequence and cause DNA double-strand breaks [13, 14].

Three types of CRISPR/Cas systems have been developed, however, type I and type III entail multiple Cas proteins as a condition to form a complex, while type II requires only Cas9 protein, which is more convenient to use, so type II serves as the one that has been used in many research applications [15-17]. The CRISPR/Cas system can design different guide RNAs for different DNA sequences without constructing proteins.

# 3. Application of plants based on genome editing technology

## 3.1. Application of ZFNs technology in plants

Applications of ZFNs in plant gene editing ZFNs were originally introduced into yeast or animal embryonic cells, mainly by transformation or embryo injection, and expressed in cells for gene editing. In plants, Wright et al. constructed tobacco protoplasts fused with the GUS: NPT II gene (600 bases missing) in 2005, and then co-introduced ZFNs, which can recognize the GUS: NPT II gene sequence, and donor DNA (600 bases) into the protoplasts by using electroporation, as a result of which homologous recombination occurred in more than 10% of the protoplasts, with 20% of the GUS: NPT II gene sequence being homologous recombination [18]. Of these, 20% of the GUS:NPT II genes were repaired by homologous recombination. This study demonstrated the potential application of ZFNs in plant gene editing, and the substantive breakthrough of ZFNs's application in plants was the knockout of the intellectuals gene SuRA in tobacco by Maeder et al. in 2008, and Shukla et al. successfully inserted the herbicide resistance gene into the endogenous maize gene IPK in 2009, where 600 herbicide resistance genes were obtained [19, 20]. 600 herbicide resistant healing tissues were obtained and the expected mutation was detected. In 2013, Curtin et al. investigated the mutagenic effects of the ZFNs technique on nine genes of the soybean genome using the root hair transformation system, and found that seven genes were mutated. As a polyploid species, the soy genome is highly repetitive, and this study suggests that the ZFNs technique can also be used for highly repetitive bases.

## 3.2. Application of TALEN to plants.

Compared to ZFNs, TALENs have advantages in terms of module construction and cost, and are more widely used in plants. Cermak et al. constructed TALENs modules by Golden Gate assembly method in 2011 to knockdown ADH1 gene in Arabidopsis protoplasts [21].

Cermak et al. used the Golden Gate assembly method to construct TALENs module to knockdown ADH1 gene in Arabidopsis protoplasts in 2011, which demonstrated that TALENs have the function of targeted mutagenesis in plant cells [21]. Li et al. used TALENs technology to disrupt the promoter sequence of the rice disease susceptibility gene Os11N3 in 2012, which improved the disease resistance of rice [22]. Zhang et al. edited the SurA and SurB genes of tobacco with TALENs technology in 2013, and they transferred the TALENs into the TALENs module [23]. In 2013, Zhang et al. used TALENs to edit the SurA and SurB genes in tobacco, and they introduced TALENs vector and donor DNA into tobacco protoplasts, and donor DNA markers were detected in 4% of the healing tissues [23]. 2015, Shan et al. edited the OsBADH2, OsCKX2, and OsDEP1 genes of rice by using TALENs, and the mutation rates were 9.7%, 25.6%, and 9.2%, respectively, with the three genes containing mutations at the same time [24]. The number of plants with mutations in all three genes was 1.9 percent..The above studies demonstrate the high editing efficiency of the TALENs technique for both single and multiple mutations in plants.

## 3.3. Application of CRISPR/Cas9 to plants 3.

In comparison to ZFNs and TALEN, the CRISPR/Cas system vector construction is preferred for its simplicity. The Cas9 gene is the same, only the sgRNAs but not the nucleases need to be designed and assembled for different target genes, so it is widely used. CRISPR/Cas9 has a wide range of potential applications in disease resistance, stress resistance, crop yield enhancement, synthesis and improvement of effective constituents in medicinal plants.

Recent studies have found that CRISPR/Cas9 is second to none in studying the function of the intrinsically disordered protein DSS1 in the response of plants to oxidative stress. The Arabidopsis genome contains two highly homologous DSS1 genes, AtDSS1 (I) and AtDSS1 (V). In order to identify the functional differences between AtDSS1s, the study generated single dss1 (I) and dss1 (V) mutant lines of both Arabidopsis DSS1 genes using CRISPR/Cas9 technology to figure out their potential roles in oxidative stress [25].

At the same time, researchers see CRISPR/Cas9 as a versatile genome-editing technique that enables multiplexed targeted gene modification for genetic enhancement of oil palm. The EgFAD2 and EgPAT genes were edited using the plant CRISPR/Cas9 vector technique by efficiently constructing multiple sgRNA expressions into single-duplex CRISPR/Cas9 vectors. Mutations found in the EgFAD2 and EgPAT target sites indicated that the Cas9/ sgRNA genome editing system effectively knocked out these two genes in oil palm and can be applied in other aspects of oil palm [26].

Moreover, CRISPR/Cas9 could be an effective diagnostic probe for plant disease treatment and prevention. In a preliminary report on the incorporation of CRISPR/Cas9 into diagnostic assays, the CRISPR-Cas9 system was used in combination with isothermal RNA amplification and a footswitch sensor for rapid and low-cost Zika virus detection. In the recent application LEOPARD (parallel RNA detection using engineered traction and target dna), the CRISPR/Cas9 system was used for multiplexed detection of multiple RNA sequences to differentiate between SARSoV-2 and its variant D614G at a single-base resolution level in patient samples [27].

## 4. Conclusion

One of the great examples of a technique used to explore the understanding of biological phenomena is genome editing, a popular and essential tool in the field of life sciences. In addition to different site-directed nucleases used for genome editing more than a decade ago, the CRISPR/Cas-based approach to genome editing has become an advanced tool due to its simplicity, ease of access, cost, and flexibility. Genome editing in plants has come a long way, with technology constantly iterating and facing challenges as well as prospects. Even the state-of-the-art CRISPR/Cas9 has its uncertainties, such as off-target activity and the stability of the transformation. Similarly, advances like precision gene editing, promoter bashing, and methylome-editing hold a promising future.

## References

[1] Shan QW, Wang YP, Chen KL, et al. Rapid and efficient gene modification in rice and Brachy podium using TALENs [J].Mol Plant, 2013, 6(4):1365-1368.

[2] Karginov FV, Hannon GJ.The CRISPR system: small RNA-guided defence in bacteria and archaea [J].Mol Cell, 2010, 37(1):7- 19.

[3] Jing Runchun, Huang Qingyang,Zhu Ying. Application of positional cloning in isolation of plant genes [J]. Genetics, 2000(03):180-185.

[4] MAO Peipei, LIU Ruixia, ZHAO Yunyun. Positional cloning of Arabidopsis genes [J]. Biological Bulletin, 2007(12):11-14.

[5] Yan Qitao, Lu Hui, Mao Wanxia et al. Map cloning technology for plant gene isolation [J]. Molecular Plant Breeding, 2005(04):585-590.

[6] LI Liwen, ZHU Yanming, LI Jie, et al. Progress of RNAi technology in plant functional genomics [J]. Journal of Northeast Agricultural University, 2007(01):119-124.

[7] ZHANG Gong, LIU Lifeng, MA Zhiying. RNA interference and its application to plant antiviral [J]. RNA interference and its application to plant antiviral [J]. Chinese Agronomy Bulletin, 2007(01):42-45.

[8] BAI Xiang,YANG Guoshun,CHEN Shi et al. Characteristics of plant RNAi and its application [J]. Biotechnology Bulletin, 2009(08):6-10.

[9] Shan Qiwei, GAO Caixia. Recent research progress in plant genome editing and derived technologies [J]. Genetics, 2015, 37(10):953-973.

[10] S. Durai. Zinc finger nucleases: custom-designed molecular scissors for genome engineering of plant and mammalian cells [J]. Nucleic Acids Research, 2005, 33 (18).

[11] J. D. Sander, P. Zaback, J. K. Joung, D. F. Voytas and D. Dobbs. Zinc Finger Targeter (ZiFiT): an engineered zinc finger/target site design tool [J]. Nucleic Acids Research, 2007. 35.

[12] A. J. Bogdanove, S. Schornack and T. Lahaye. TAL effectors: finding plant genes for disease and defence [J]. Current Opinion in Plant Biology, 2010, 13 (4).

[13] H.S. Xu, P.D. Li, F.M. Dai, et al. Research progress of genome editing technology and its application in plants [J]. Anhui Agricultural Bulletin, 2015, 21(18):15-19.

[14] Arrangou R.CRISPR- Cas systems and RNA- guided interference [J].Wiley interdiscip Rev RNA, 2013, 4:267-278.

[15] Jinek M, Chylinski K,Fonfara I, et al. A programmable dual RNA-guided DNA endonuclease in adaptive bacterial immunity [J].Science, 2012, 337:816-821.

[16] Cong L,Ran FA,Cox D,et al. Multiplex genome engineering using CRISPR/Cas systems [J].Science,2013,339:819-823.

[17] Mali P, Yang L,Esvelt KM,et al. RNA-guided human genome engineering via Cas9 [J].Science,2013,339:823-826.

[18] Wright D A,Townsend J A, Winfrey R J Jr,et al.High-frequency homologous recombination in plants mediated by zincfinger nucleases [J].Plant,2005,44:693-705.

[19] Maeder ML, Thibodeau- Beganny S, Osiak A,et al. Rapid ‘‘open- source’’engineering of customized zinc finger nucleases for highly efficient gene modification [J].Mol Cell,2008,31:294-301.

[20] Shukla V K, Doyon Y,Miller J C,et al. Precise genome modification in the crop species Zea mays using zinc-finger nucleases [J].Nature,2009,459:437-441.

[21] Cermak T,Doyle EL,Christian M,et al.Efficient design and assembly of custom TALEN and other TAL effectorbased constructs for DNA targeting [J].Nucleic Acids Res,2011,39:e82.

[22] Li T, Liu B,Spalding MH,et al.High-efficiency TALEN-based gene editing produces disease- resistant rice [J].Nat Biotechnol, 2012,30:390-392.

[23] Zhang Y,Zhang F,Li X,et al. Bogdanove AJ, Voytas DF Transcription activator-like effector nucleases enable efficient plant genome engineering [J].Plant Physiol,2013,161:20-27.

[24] Shan Q, Zhang Y, Chen K,et al. Creation of fragrant rice by targeted knockout of the OsBADH2 gene using TALEN technology [J].Plant Biotechnol,2015,1-10.

[25] Nikolić I, Samardžić J, Stevanović S, Miljuš-Đukić J, Milisavljević M, Timotijević G. CRISPR/Cas9-Targeted Disruption of Two Highly Homologous Arabidopsis thaliana DSS1 Genes with Roles in Development and the Oxidative Stress Response [J]. Int J Mol Sci. 2023 Jan 26; 24(3):2442.

[26] Bahariah B, Masani MYA, Fizree MPMAA, Rasid OA, Parveez GKA. Multiplex CRISPR/Cas9 gene-editing platform in oil palm targeting mutations in EgFAD2 and EgPAT genes [J]. J Genet Eng Biotechnol. 2023 Jan 11; 21(1):3.

[27] Vats S, Kumawat S, Kumar V, Patil GB, Joshi T, Sonah H, Sharma TR, Deshmukh R. Genome Editing in Plants: Exploration of Technological Advancements and Challenges [J]. Cells. 2019 Nov 4; 8(11):1386.