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A Comparative Review on Genome Editing Approaches

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Abstract: Genome editing is a precise modification of the genome in many organisms using engineered nucleases as an emerging and powerful technique. All genome editing tools are relied on creating double-stranded breaks (DSBs) at the target locus followed by subsequent repair through either homology-directed repair (HDR) or non-homologous end-joining (NHEJ) pathways which are capable of producing desired genetic modifications. The main genome editing tools include zinc finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs), and CRISPR/Cas9 system. By creating precise genotype modifications, these tools can create different phenotypes in various sciences, especially medicine, biological research, and biotechnology. Genome modification in model organisms has been possible with the emersion of TALENs since 2010. Then, in 2013, the CRISP/Cas9 system caused the beginning of a new era of genome editing research, which can be considered a revolution in biology. Furthermore, in the near future, genome editing will be able to treat the treatment of genetic diseases. In addition, the prospect of genome editing in producing different crops and livestock with useful characteristics is also promising. These products are known as edited crops that are not genetically modified organisms (GMOs). In this review, the main genome editing tools will be introduced and compared briefly.

Keywords: genome editing; zinc finger nucleases (ZFNs); transcription activator-like effector nucleases (TALENs); CRISPR/Cas9 technology.

1. Introduction

Genome editing is used to make precise genetic modifications for various purposes, such as studying the gene function, biological mechanisms, and pathology of diseases. Despite the random mutagenesis methods, which create unpredictable phenotypes, these methods have enabled researchers to create desired changes in a target gene. The intended changes include target gene mutagenesis, insertion and replacement, suppression or activation of the gene expression, and target chromosomal rearrangement. Generally, genome editing depends on creating specific double-stranded breaks (DSBs) in the target gene by using engineered endonucleases followed by a final repair of these double-stranded breaks through homology-directed repair (HDR) or non-homologous end-joining (NHEJ), creating a desired genetic modifications [1-3]. The HDR pathway normally uses the inserted nucleotides with regard to the sister chromatid DNA as the template for repairing the break. On the other hand, in NHEJ, small insertions or deletions lead to a change in the open reading frame (ORF) and, ultimately,

gene destruction [4]. Three main classes of endonucleases, including zinc finger nucleases (ZFNs) [5], transcription activator-like effector nucleases (TALENs) [6], and CRISPR/Cas9 system [7] are known as the main tools in genome editing.

2. Zinc Finger Nucleases (ZFNs)

Miller discovered the first zinc finger motifs with specific DNA binding affinity as part of the transcription factor TFIIIA in Xenopus in 1985. Nevertheless, ZFNs have a shorter history, so 15 years after Miller, the first zinc finger nuclease was discovered [8]. The Cys2 Hys2 is the human genome's most abundant DNA binding motif. In this motif, each zinc finger domain includes 30 amino acids, consisting of two non-parallel beta sheets against an alpha helix and one zinc atom, which makes a non-covalent bond with two cysteines and two histidines (Cys2- Hys2) [9]. In ZFNs, the DNA binding site includes three or four modules with a zinc finger structure. An alpha helix in each zinc finger domain identifies a specific triplet sequence of DNA [10]. Thus, a target site with three modules is able to recognize the base pair. Also, due to the modularity feature, the zinc finger domains can be designed in a targeted way for binding to predetermined regions of the DNA sequence in the desired genome site [11]. Nevertheless, the synergistic effects of adjacent modules on each other complicate the prediction of the real efficiency of each designed ZFN [10]. The ZFNs motif includes a zinc finger protein attached to the cleaving domain of the Fokl restriction enzyme, which was initially named as a chimera restriction enzyme and then ZFNs (Figure 1). Fokl enzyme was discovered in 1981 and functions as a homodimer [12]. The domains of the Fokl enzyme include detection and cleaving domains that create the excision at the target site [13].

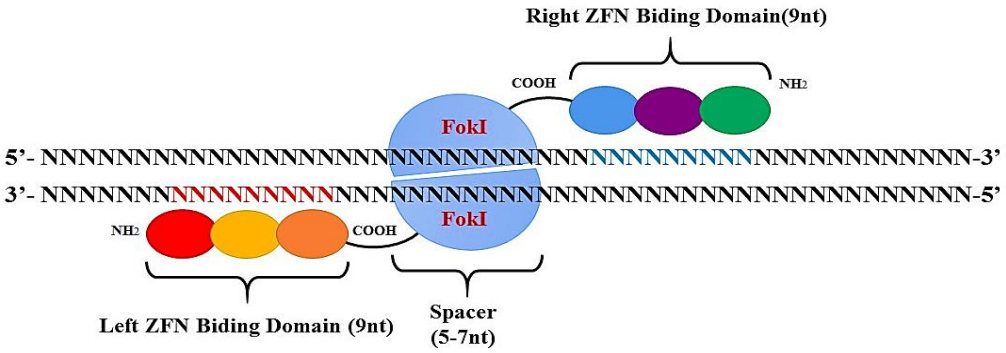

Figure 1. ZFN function; FN, as ZFN, contains right and left monomers that each typically includes three to four zinc finger proteins (ZFPs) and Fokl restriction enzyme cutting the DNA during dimer formation. Each ZFP identifies a specific triplet sequence of DNA.

Eukaryotic cells choose two pathways for repairing the break after ZFN-mediated excision. The first pathway is HDR, concerning that the enzyme-induced cleavage leads to the loss of some nucleotides in the cleavage region. The missing sequences in the damaged region are completely reversed by a copy-and-paste mechanism using the inserted information in the sister chromatid DNA [14,15]. The second pathway is NHEJ, which is error-prone and often results in short insertions or deletions (indels) of a few base pairs (10-20 base pairs) in the break region. In plants, the frequency of the repair pathway HDR is 10 times less than the NHEJ pathway. Also, the HDR pathway occurs only during the S/G2 phase of cell division, when sister chromatids can be employed as a template to repair the damaged site. Thus, the efficiency of the HDR pathway is less than the NHEJ pathway, especially in the divided cells [16-19].

In contrast, the NEHJ pathway is active in dividing or dividing cells, and non-specifically causes indels in genes and ORF disruption. Also, the main components involved in the NHEJ pathway include Ku DNA-PKcs, Pol complex, Pol mu Artemis: DNA-PKcs lambda, and polynucleotide kinase (PNK), and in the HDR, include RecA and Rad51. Genome editing can also act through base substitution, as well. Precise and efficient point mutations can be created in the target site. In the base editing system, cytidine deaminase is integrated into dCas9 by replacing cytosine with thymine. There is no need for DSBs and donor template strands in this recently-created system. Therefore, it has a high potential for gene modification in plant and animal cells [20-23].

In vertebrates, around 20% of protein-encoding genes contain C2H2 domains, which have the ability to bind a wide range of nucleotide sequences [24]. Thus, these domains are potential targets for designing DNA-binding proteins that bind to the desired predefined sites. It has been found that ZFs can be engineered to make specific changes [25]. Hence, various methods have been developed for engineering the ZFs binding specificity. Also, an important aspect of using ZFNs is their specificity, which causes unwanted mutations, by cutting in off-target sites, leading to toxicity [26]. In addition, zinc finger domains have a limited capacity to target each sequence of DNA due to the dependent effects on the external and internal content of the adjacent fingers, affecting efficiency and specificity [27,28].

2.1. ZFNs specificity.

Generally, not only can DNA-binding proteins bind to target sites with a high affinity, but also they can bind to similar or off-target sites with lower affinity. Consequently, the off-target effects lead to unwanted mutations and toxicity [29]. Thus, it is very important for precise genome editing to select highly specific targeted nucleases. Because Fokl nuclease domains must be in dimer form to make a cleavage, two ZFN molecules bound to the target DNA must be at a close distance and in a suitable orientation to each other for each other the formation of functional zinc finger nucleases [30,31]. Moreover, the specificity largely depends on the proper linkage between two ZFNs and half-sites with a suitable orientation and distance from each other, allowing Fokl domains to dimerize and cut their in-between sequence [32]. Various methods have been developed to enhance the specificity and reduce toxicity. For example, using multiple zinc finger arrays can lead to DNA binding with higher affinity [33]. In a study in which target and off-target cleavages were created with three and four zinc finger pairs, it was found that ZFN with four finger pairs can create a higher target and lower off-target cleavages [34]. However, more domains cannot always guarantee greater specificity. For example, a high amount of off-target cleavage was observed in mouse cells with ZFNs with five finger pairs [35]. Changing the interface length between zinc finger domains and the Fokl domain leads to altered spatial requirements in the half-site, which can also influence the occurrence of the on- and off-target cleavage sites. In addition, variants of the Fokl enzyme, forming a heterodimer structure and unstable homodimers, can be created to minimize the off-target cleavages [36,37]. It has been found that homodimers disproportionately show activity at off-target sites. Thus, a proper strategy to increase the specificity is preventing homodimer formation [38]. Another option for reducing off-target activity is using enzymes that only create nicks. It has been shown that ZFNickases can stimulate the HDR pathway in the target site. In this state, with the enzymatic activity of one of the monomers, a nick is created, whereby the cell mostly uses a homologous recombination mechanism for its repair [39]. In zinc finger

requirements, the catalytic activity of one of the enzyme domains has been lost, though it does not affect the dimerization process [11].

2.2. ZFs designing methods.

There are various methods for designing and constructing zinc finger proteins. Modular assembly is the simplest method in that specific zinc finger domains are constructed separately for each triplet sequence and then bind together [40]. This method is based on the fact that each module binds its detection site independently from the adjacent modules. This allows the production of separate fingers, which can bind 64 possible triplets. Nevertheless, ZFNs based on the modular assembly have poor editing activity and high toxicity. Furthermore, the best ZFs may be lost at the time of their selection from the random library due to dependence on the context [41,42]. Thus, the context-dependent assembly (CoDA) method was developed to prevent this issue. In this method, two double-finger units such as F1F2 and F2F3, common to each other in F2, are used to make CoDA arrays. These units are selected from the ZiFDB database (http://zifdb.msi.umn.edu/) [43]. For example, the F1F2 CoDA unit detecting the sequence (GAGGGG) 3 binds the F2F3 CoDA unit detecting the sequence GGGGTG 3, leading to the development of a triplet finger array which detects the new sequence (GAGGGGGTG-3) [44].

3. Transcription Activator-Like Effector Nucleases (TALENs)

In nature, TALEs are transcription factors in plant pathogenic bacteria Xanthomonas genus, consisting of three main parts: N-terminal region (NTR), C-terminal (CTR), and DNA-binding domain (DBDs), containing multiple interlinked repetitions. The sequence of the N terminal has secretory peptides, while the sequence of the C terminal has nuclear localization signal (NLS) peptides (Figure 2). These peptides are required for transferring to the nucleus, where TALEs bind their target site and regulate the transcription through the effector domain (ED). TALEs proteins enter the host cells through the secretory system of type III bacteria, bind to the DNA-specific sequence, and enhance the expression of some host genes, enabling pathogen development [45-47].

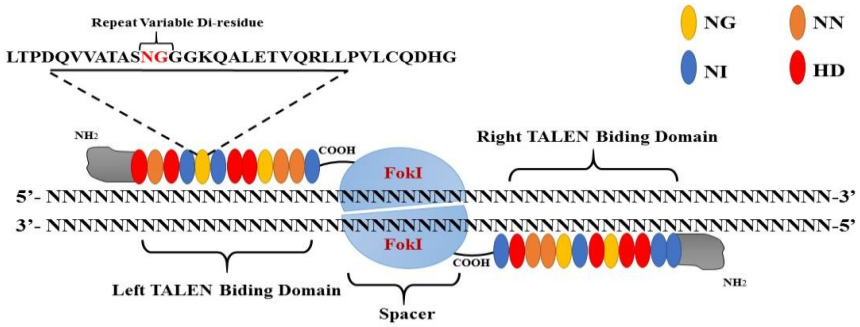

Figure 2. TALEN structure; which consists of right and left monomers of TALE proteins and Fokl restriction enzyme cutting DNA during dimer formation. In this system, each TALE protein detects a single specific sequence of DNA.

The DNA recognition mechanism by TALE proteins was discovered in 2009 by in silico analysis using a large set of DBDs sequences and their target sequences. The target detection involves two key amino acids in positions 12 and 13 of each unit, containing 34 amino acids. These positions are variable among repeated units and known as repeat variable di-residue (RVD) [48,49]. At least 23 RVDs have been identified that are more common for

NG NI, NN, and HD, specifically identifying A, T, G, and C nucleotides, respectively. The specificity of the four RVDs causes it to be applicable for constructing artificial TAL effectors in order to target the desired sequence. In genome editing, the TALENs approach is a type of combinatorial protein including the TAL effector and Fokl endonuclease cutting domain as a dimer, which is used to cut at the target site. Each repeat in TALENs has 30-35 amino acids, which can detect a special nucleotide, and the last repetition has only 20 amino acids, known as half repeat. The size of TALENs construction is typically large (more than 5K base pairs), which causes a restriction in terms of placement in specific vectors [50,51].

3.1. TALENs specificity.

Many studies have reported that TALENs show more editing activity and less toxicity, in other words, more specificity and less off-target effects than ZFNs. Each repeat interacts with one base in this system, and its specificity is determined by RVDs [52]. Since Fokl functions as a dimer, binding the two TALENs to two half-sites with a suitable orientation and distance can enhance specificity. Also, off-target effects can be reduced by producing TALENickases by creating Fokl heterodimers which contain a mutated monomer in the catalytic site [53].

3.2. TALENs synthesis.

The method used for TALENs synthesis is simpler than those used for TALEs detection codes and does not depend on any context-dependent effects. The simple one-to-one bound between each RVD and the desired nucleotide allows TALE domains to be designed easily [54].

3.2.1. Ligation based on Golden Gate cloning.

This method uses an archive of TALE repetitive units, type II restriction enzymes with different cutting and binding sites, and DNA ligase. The combination of these enzymes and a suitable set of plasmids can bind 2-10 repetitions in a reaction with a defined order. Among various methods of TALENs synthesis, this method is simple, rapid, and inexpensive and is used by many researchers [55].

3.2.2. PCR-based ligation via Golden Gate.

In this method, repetitive units are proliferated by using four plasmids. Each plasmid encodes one of the repeated units of NG, NI, HD, and NN, which specifically detect nucleotides A, T, G, and C, respectively, and are amplified by suitable primers. The specificity of four RVDs makes them suitable for synthesizing artificial effector TAL to target the desired sequences. The amplified pieces are purified and then ligated in the first stage through the Golden Gate cloning method. The ligated pieces are amplified again by PCR and used in the second ligation stage. This method requires less plasmid and time than cloning-based ligation [56,57].

3.2.3. Restriction enzymes assembly Ligation (REAL).

In this method, the first two repetitions of TALE and then a set of double repetitions are bound together, which continues further in this way. This method is the simplest method of

TALENs ligation, but it is longer due to the numerous stages of cloning. Double-quadrupole prefabricated modules are used in the improved state of this method, known as REAL-Fast, instead of using single units [58].

3.3.3. Fast Ligation-based Automatable Solid-phase High-throughput (FLASH) system.

The repetitions used in this method are almost the same as the REAL-Fast method. The main difference is in the ligation of the repeated units, which is performed on magnetic grains. In this method, a biotin-labeled unit and a quadruple prefabricated module first bind to each other and hang off streptavidin-coated magnetic grains. Thereafter, purification, cutting, and serial ligation are performed to assemble TALEs [59].

4. CRISPR/Cas9 system

This system, which was discovered for the first time in the genome of Escherichia coli [11], is an acquired immunity mechanism in various bacteria and archaea against foreign agents, including plasmids and viral genomes [60]. CRISPR/Cas system is categorized into three major classes (I, II, and III), with a gene family encoding specific Cas enzymes and a special functional mechanism. Types I and III utilize various Cas enzymes for endonuclease activity, whilst type II employs only Cas9 enzyme for this purpose [61]. From an evolutionary point of view, the rate of changes and evolution in the structural and functional diversity is very high due to the constant competition between the parasite and the host in the CRISPR/Cas9 system. Accordingly, in recent classification, the CRISPR system is classified into two classes (I and II) and also six types (I-VI) [62].

Most genome engineering studies have utilized CRISPR system type II, derived from *S.pyogenes*, SpCas9 bacterium. The advantage of system type II is the dependence on a protein (Cas9) for nuclease activity.

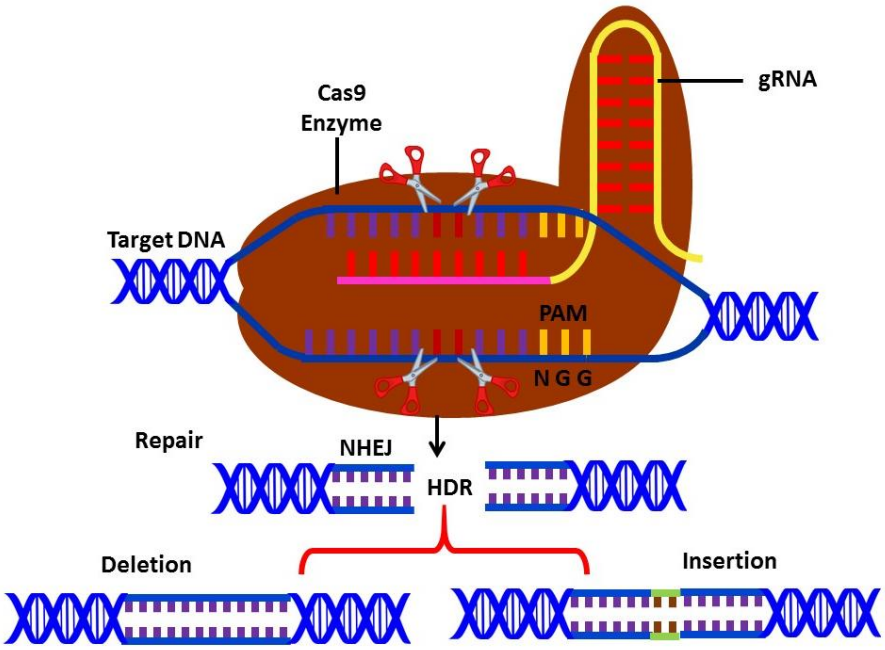

Figure 3. The sgRNA complex structure binds the target DNA (SpCas9 - gRNA complex); first, the PAM sequence is detected in the pathogen genome, and then the DNA is edited by a double-stranded DNA break. In this system, the PAM sequence is downstream of the target sequence, and the Cas9 nuclease cuts the genomic sequence, which is immediately located on the 5' site of the PAM sequence.

Also, system II requires some kinds of RNAs, such as crRNA, pairing with the target genome sequences and functioning as a Cas guide, and tracrRNA, which acts as a crRNA trans activator for maturity and summoning up Cas9 to the desired site [63,64]. TracrRNA and crRNA sequences have been integrated into a chimeric sequence, known as small guide RNA (sgRNA), containing the characteristics of both types of RNA, for use in genome editing (Figure 3) [65]. Also, the CRISPR/Cas9 system can be used for treating genetic diseases. For example, the mutated site in the dystrophin gene leading to Duchene's muscle dystrophy disease (DMD) has been eliminated by CRISPR. Then the expression of dystrophin returned to a normal level, and the muscular functions were significantly improved. All CRISPR sites contain tandem repeats and spacers. Tandem repeats include identical and spacer sequences originating from the genome of foreign agents [66-68]. CRISPR sites associated with proteins (Cas) can make an acquired immunity against invading DNA. If a microorganism survives the pathogen, the CRISPR system will be able to insert a fragment of the invading DNA into the genome and use it to deal with future attacks [69,70].

Briefly, the immunity in bacteria via the CRISPR system is operated in three stages: Admission: In this step, short fragments of virus or plasmid DNA are detected and inserted as a spacer between two adjacent repeats into the CRISPR sites [71]; Expression: During this step, CRISPR sites are copied as a Pre-crRNA, which contains a complete complex of CRISPR repeats and embedded sequences derived from invasive agents among them [72]; Interference: In this step, Pre-crRNA is separated into crRNA as the small guide sequence by a special endoribonuclease [73].

4.1. Cas9 protein structure.

Characterizing the crystalline structure of the Cas9 protein in *S. pyogenes*, was helpful in understanding the interaction between the components of the complex, including Cas9, crRNA, tracrRNA, and the target DNA at the molecular scale. Generally, Cas9 protein includes a nuclease lobe (NUC) and a recognition lobe (REC). The REC lobe, which consists of a long alpha helix and REC1 and REC2 domains, is considered the specific functional domain of Cas9. At the same time, the NUC lobe is composed of RuvC HNH and PAM-interacting (PI) domains [74]. HNH and RuvC domains cut the target sense and antisense strands, respectively, and create a DSB upstream of PAM [75]. Also, the PAM sequence can regulate the sgRNA recognition control system [76]. If each nuclease domain is inactivated, Cas9 can only cut one strand as a nickase [77].

4.2. Specificity of the cleavage.

Although specificity in targeting depends heavily on the gRNA sequence, the Cas9 protein nuclease activity in the CRISPR-Cas9 system is dependent on the PAM-specific sequence. In this system, Cas9 nuclease can cut any genomic sequence that is immediately located on the 5' side of the PAM sequence, and in other words, the target sequence is cleaved in downstream [78]. The absence of a PAM sequence can change the affinity between Cas and the target DNA, and therefore, the specific sequence can help to distinguish non- -target sequences from off-target sequences [79,80]. PAM sequence in *S. pyogenes* (SpCas9) is 3'-NGG-5', and in *S. thermophilus* (StCas9) is 3'-NNAGAAW-5' [81,82]. The SpCas9gRNA complex first detects the PAM sequence in the genome, and then DNA-RNA base pairs are formed by opening the double-stranded DNA. The detecting sequence of the crRNA region is

generally 20 nucleotides long, but fewer or more base pairs can increase the specificity. Therefore, the sequence of 20 bp in gRNA and 3 bp in PAM can increase the specificity of the CRISPR/Cas9 system. Nevertheless, weaving the 3'-NGG-5' 5 motif causes restrictions in some cases, as in the regions of the genome with high AT sequence [83,84].

4.3. Off-target effects.

Off-target mutagenesis is one of the main problems in the CRISPR-Cas9 system, especially in gene therapy [85]. Nevertheless, this issue does not seem to be serious in plants, which can be due to transformation efficiency, gene expression level, and codon usage bias (CUB) in plants. Off-target effects are defined as the acceptance or tolerance of Cas9 to awkward sequences in the sgRNA. Off-target effects could be more likely when there is a high similarity of gRNA sequence with an off-target site up to three bps [65]. Various studies show that the mismatched bps at the end of the 3′ target sequence (usually 8 to 14 bps upstream of the PAM sequence) are less tolerated, while the mismatched bps at the end of the 5′ target region are better tolerated than 3′ target sequence and they are more acceptable [86]. Moreover, the value of gRNA and its ratio to Cas9 also affect the off-target effects [87].

In general, although Cas9 enzyme has different applications because of its high nuclease activity as well as wide targeting range, it has limitations due to high molecular weight and off-target effects. Nevertheless, some mutations occur in Cas9 variants, such as eSpCas9 and SpCas9-HF [88], which reduce nonspecific interaction between Cas9 protein and the target sequence. In addition to various strategies to reduce off-target effects, there are numerous laboratory methods, such as digenome-seq, GUIDE-seq, and HTGTS, to identify off-target sites [89].

4.4. Decreasing off-target effects in the CRISPR/Cas9 system.

4.4.1. Selection of GUIDE-Seq with minimum potential sites.

Off-target is detected by whole genome homology search. Among them, some sequences are chosen whereby the mismatched pairs are concentrated in the region close to PAM since they are tolerated less for Cas9 functioning [90].

4.4.2. Selection of truncated length GUIDE-Seq (trui-gRNA).

In this solution, gRNA with 2-3 nucleotide size is truncated at 5'. It has been found that the selection of shorter sequences (17 or 18 nucleotides) reduces the editing efficiency to a small extent while significantly reducing undesired mutations, thereby mitigating off-target effects [89].

4.4.3. Use of the pair need strategy (Cas9n).

Through inactivating one of the Cas9 domains, a variant has been created that functions as a demand. Using a pair of closely related sgRNAs, Cas9n can create two adjacent splices in single strands, consequently, a DSB. This solution reduces the off-target activity and can increase specificity by up to 50-1500 times. The pair need strategy has caused gene deletion in mouse eggs without reducing the cutting activity of the target sites [91,92].

4.4.4. dCas9-Fok strategy.

Dead-nuclease Cas9 (dCas9) is created by inhibiting the enzymatic activity of both RuvC and HNH domains. DSB is produced by combining the Fokl nuclease domain with dCas9 as a dimer and selecting a pair of sgRNA with suitable orientation and distance. Investigations have shown that this method significantly enhances specificity [93].

4.5. CRISPR/Cas12a system.

The Cas12a protein was known as an important discovery in 2015 at Feng Zhang's laboratory, one of the pioneers of the CRISPR system. Cas12a protein is the smaller and simpler version of Cas9 belonging to class II and type V. This protein is isolated from *Staphylococcus Aureus* and has a higher editing activity than Cas9. The small size of this protein has made it easier to transfer it into cells and tissues. Furthermore, the specificity of Cas12a endonuclease is high, and thus its off-target effects, especially in human cells, are negligible [94,95].

Some differences between Cas12a and Cas9 have caused the CRISPR/Cas12a system to overcome some limitations of the CRISPR/Cas9 system. For example, the Cas9 protein requires two molecules, crRNA, and tracrRNA, while Cas12a needs one RNA molecule (crRNA) for cutting DNA [96]. Cas9 includes RuvC and HNH nuclease domains and cleaves both DNA strands at the same site, causing blunt ends, while, Cas12a only contains the RuvC nuclease domain, which creates two sticky ends at the target sites by cutting only one strand [97]. The Cas12a protein cuts DNA at different sites and provides more options while selecting a site for editing. The cleavage and detection sites in Cas9 are close together, while in Cas12a, they are further apart. The PAM sequence in Cas9 is rich in '5-NGG-'3, while in Cas12a, it is rich in T (5'-TTTN-3) [65].

4.6. CRISPR transfer methods.

According to the specific, precise, and effective editing, particularly in gene therapy, transferring the CRISPR system into the cells is highly important. The CRISPR construction transfer can be performed as DNA, mRNA, or ribonucleoprotein. The transformation strategies can also contain microinjection, electroporation, and viral and non-viral vectors such as nanoparticles, liposomes, and agrobacterium-mediated vectors [98-101].

5. Comparison of ZFNs, TALENs, and CRISPR/Cas9 systems

ZFNs and CRISPR/Cas9 systems are based on DNA-RNA interaction and easily designing RNAs for each specific sequence. Nevertheless, ZFNs and TALENs are based on protein-DNA interaction, so they need to be reconstructed for each target DNA sequence. This is the most important advantage of the CRISPR/Cas9 system [102]. Other benefits of the CRISPR/Cas9 systems are: The simplicity of the 20-base pair sequence in sgRNA can be easily designed for targeting any new DNA sequence [65]; Several sgRNAs can function associating with a Cas9 protein simultaneously on several different sites, which is one of the most important advantages of this system compared to TALENs and ZFNs. This potential has been used in rice and Arabidopsis for removing large chromosome fragments containing several genes. In addition, concurrent targeting of several genes improves several traits in agricultural crops and can also be used in applied research to understand the role of each gene in a complex

network [103,104]; The CRISPR/Cas9 system is able to edit highly epigenetic-regulated genomic loci. This is especially important in plants where around 70% of CpG/CpNpG sites, especially CpG islands, have been methylated in the promoter of their proximal exons. Thus, the CRISPR-Cas9 system is suitable for genome editing in plants, especially for Monocotyledons such as rice, whose genome contains large amounts of GC [105,106].

Table 1. Comparison between genome editing systems

System	ZFNs	TALENs	CRISPR
Function	Cleavage based on DNA:protein interaction	Cleavage based on DNA:protein interaction	Cleavage based on DNA:RNA interaction
Nuclease designing and assembly	Hard and costly	Mostly possible in the laboratory but highly difficult	Easy
Designing efficiency	Low	High	High
Assembly efficiency	Variable	High (%99<)	High (%90<)
Targeting range	Restricted, depending on ZFs	Unrestricted independence on PAM	Restricted by PAM but not generally
Off-target effects	Yes	Yes	Yes
Sensitivity to DNA methylation	Undefined	Sensitive to CpG methylation	Non-sensitive to CpG methylation
High operating power	No	Restricted	Possible

6. Conclusions

The discovery of different genome editing tools such as TALENs, ZFNs, and CRISPRs systems allows precise and targeted genome modification in many organisms and tissues. In addition to research in the medicine and pharmaceutical industry, genome editing technology can provide better nutrition and food safety in agriculture by improving crops. Nevertheless, despite the powerful features of CRISPR in genome editing, this system has some limitations. The most important challenge in this field is the off-target effects. The discovery of the new protein Cpf1, which has higher editing activity compared to Cas9, led to enhancing the accuracy and efficiency of the system. Thus, ongoing research is on producing newer and more efficient versions of Cas to elevate the accuracy and reduce off-target effects. In this regard, predesigned versions with higher efficiency may be achieved through protein engineering, although no reports have been published. Another existing challenge is the increased ethical concerns caused by using CRISPR technology. Thus, it is necessary to establish rules for applying genome editing methods in medicine and applied research. Generally, TALENs and CRISPR/Cas9 systems have considerable advantages over ZFNs because of the one-by-one detection of nucleotides, leading to their design and structure simplicity. Further, the potentials of the CRISPR/Cas9 system have been clarified in three dimensions genomics, transcriptomics, and epigenomics. All these allow this method to induce or suppress a specific set of genes simultaneously while also supporting epigenomic reprogramming concurrently.

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Conflicts of Interest

The authors declare that there is no conflict of interest. The funders had no role in the study's design; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

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