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Review article

Application of the CRISPR/Cas9 Gene-editing System and Its Participation in Plant and Medical Science

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human diseases

Abstract

The long-term goal of scientists and breeders is to study the efficacy of a gene, as well as to use it in the development of human life and in the development of improved quality and varieties of crops. CRISPR/Cas9, a gene-editing tool, has already uncovered a wide range of applications in areas such as human disease diagnosis and the development of new crop varieties. This review provides basic ideas about CRISPR/Cas9 as well as its importance in the current context. CRISPR/Cas9 editing tool has more contributions to plant science than medical science. As a mature cutting-edge biotechnological technique, CRISPR/Cas9 has been applied in a variety of crop related research and development areas including disease resistance, plant development, abiotic tolerance, morphological development, secondary metabolism, and fiber formation. Lastly, some of the limitations of this system have been mentioned, and aspects of more research in the future have been suggested. Through this review, readers will better be able to understand the CRISPR/Cas9 genome editing system and will be familiar with much of the research that has occurred from the past to the present in a range of science fields.

1. Introduction

CRISPR/Cas9 is a novel technology that allows biotechnologists and research scientists to edit sections of genetic material by destroying and/or adding/changing DNA sequences. In the scientific world, the most straightforward, reliable, and beneficial way of genetic modification is creating quite a stir [1]. The full name of CRISPR is “Clustered regularly interspaced palindromic repeats”. With this technology, it is relatively easy to correct errors in the genome and thus change the functions of genes in organisms [2]. CRISPR/Cas9 has made a lot of contributions to the biotechnological, agricultural, and medicinal fields [3]. Some bacteria have an immune system like a built-in gene editing system, akin to the CRISPR/Cas9 method, which they use to fight pathogen

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attacks. Bacteria use CRISPR to cut off portions of viral DNA and retain a small portion of it so they can identify the virus and protect themselves. This bacterial technique was modified and has been applied to different animal cells, such as mice and people. CRISPR/Cas9 has a significant contribution of curing mouse genetic disorders by using CRISPR/Cas9 to repair defective mouse DNA [4]. The tool also has a role in human embryos [5] and many medical applications such as curing infectious diseases (cancer, HIV/AIDS), gene therapy, and so on [6].

The world's population will have increased 1.5 times from the current level by 2050. The availability of water and farmland is decreasing and the food demand is predicted to increase by 30%-70% [7]. It will be necessary to increase food and crop production to feed the increasing population. In the early years of the genetics field, plants were modified by selection and breeding [8]. Without knowledge of genes, gene modification, and mutagenesis, our ancestors modified the genetic profile of animals and plants by breeding them for characteristics that would increase the yield of food [9, 10]. Over the years, several methods of plant gene editing have already been established to speed up plant breeding, make it more reliable, and make it adaptable to many different species. The newest method of genetic engineering is genome modification using programmable endonucleases. The editing of the plant genome now uses three different types of controllable endonucleases: zinc finger nucleases, transcription activator-like effector nucleases (TALENs), and CRISPR/Cas9 [11-14]. Nowadays, CRISPR/Cas9 is the most popular gene editing tool in plant science. By this system, salt [14], drought [15], and cold [16] tolerance rice varieties were developed. High-yielding maize varieties were developed with the CRISPR/Cas9 gene-editing tool [17]. The CRISPR/Cas9 system helps much in raising the yields of different crops by preventing the attack of pests and pathogens.

In this review paper, our main goal is to increase knowledge about the CRISPR/Cas9 system and its present and future research applications in the areas of plant and medical science (Figure 1). We will also go through some of the challenges and moral debates surrounding this innovative technology.

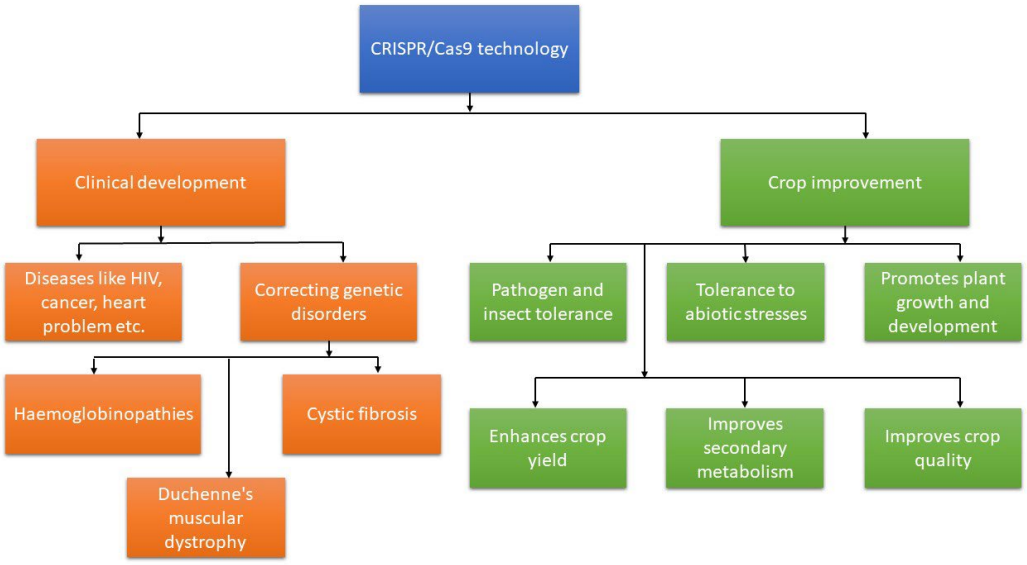

Figure 1. Overview of the role of CRISPR/Cas9 in plant and medical science

2. Overview of CRISPR/Cas9

In the CRISPR/Cas9 system, two key components are used cooperatively to modify DNA. The first is the enzyme Cas9, which is used for cutting two DNA strands at a specific location in the genome and functions as a pair of "molecular scissors" that can add or remove DNA fragments. The second component is guide RNA (gRNA), which is an individual fragment of RNA. It is composed of a small (only about 20 bases long) spacer RNA fragment that is enmeshed within a larger RNA scaffold. This component attaches to DNA, while the preplanned sequence directs Cas9 to the appropriate location in the genome. By doing this, the Cas9 enzyme is guaranteed to make a precise cut in the genome [18]. The goal of gRNA is to locate and attach to a particular DNA sequence. The complementary RNA bases in the gRNA match those in the genome's target DNA sequence. As a result, the gRNA will only attach to the target sequence and not to any other part of the genome. A cut is made across both strands of DNA by the Cas9 after it follows the gRNA to the same spot in the DNA sequence. Next, the cell attempts to repair the DNA damage after realizing that it has been damaged (Figure 2).

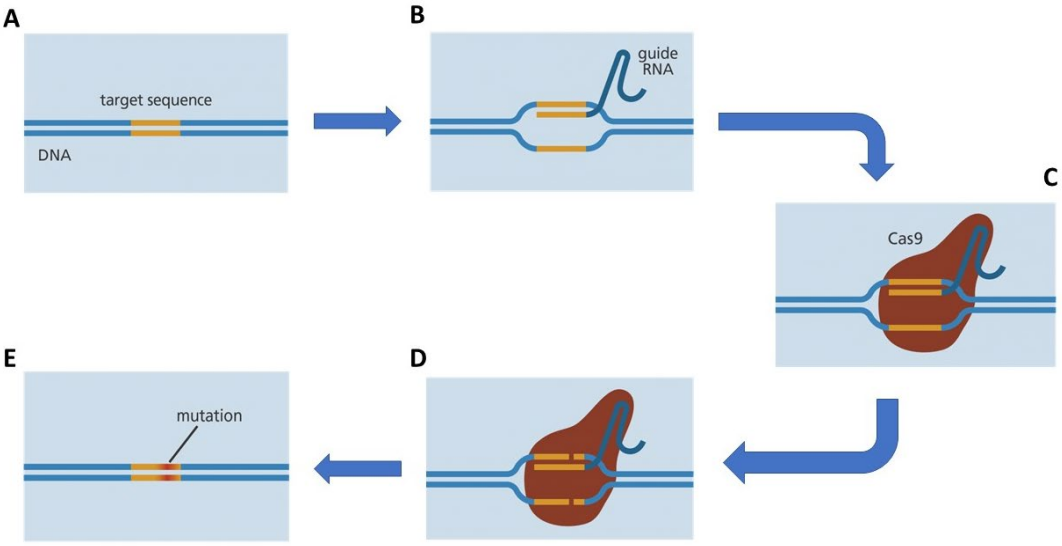

Figure 2. Working model of the CRISPR/Cas9 system
a) target sequence, b) the target sequence is bound by gRNA, c) enzyme Cas9 attaches to gRNA, d) the DNA strands are cut by the Cas9 enzyme, e) the repair of the incision results in mutation

3. Potential Uses

3.1 Use in medical science

The prevention and treatment of human diseases is a major area of focus for genome editing. Genome editing is currently employed in research facilities to study diseases in cells and animal models. It is being investigated in research and clinical trials for a wide range of illnesses, including single-gene diseases like sickle cell disease, hemophilia, and cystic fibrosis. Additionally, it shows

promise in the management and avoidance of more complicated illnesses like HIV, cancer, mental illness, and heart disease [19, 20]. With the aim of using the patient's own immune system against tumor cells, cancer immunotherapy with CRISPR has recently sparked a lot of attention [21]. The use of genetically modified T cells, or CAR (chimeric antigen receptor) T cells, which enable the targeting of tumor-associated antigens and may improve the therapy response, is one promising field of immunotherapy [22, 23]. Several crucial steps need to be followed in order to prepare functional CAR T cells (Figure 3).

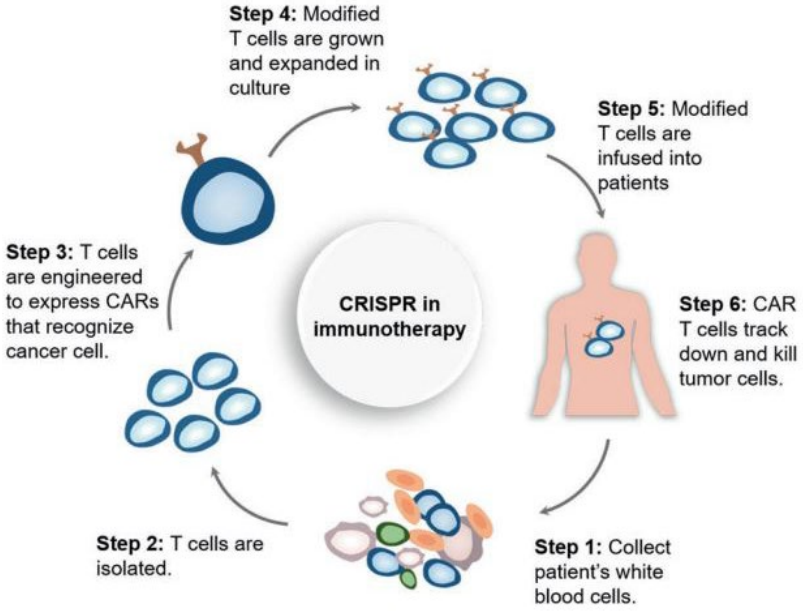

Figure 3. Genome editing technique used in the production of CAR T cells [23]

3.1.1 Correction of genetic disorder

The possibility of treating genetic disorders brought on by single-gene mutations is one of the most promising uses of CRISPR/Cas9. Haemoglobinopathies, Duchenne's muscular dystrophy, and cystic fibrosis (CF) are a few examples of such illnesses.

Nowadays, different experiments are done on mouse or rat models. The use of CRISPR/Cas9 on humans has not been established yet, but it soon will be applied in medical settings on humans. To better understand CF pathogenesis, CF models were developed in four species (rat, mouse, pig, and ferrets). Fan *et al*. [24] state that the sheep CF model may be relevant to humans because their lung anatomy and development are similar. To produce $CFTR^{-/-}$ and $CFTR^{+/-}$ lambs, they created cells with the CFTR gene disrupted. This large animal model will be an important tool for promoting the investigation and creation of novel CF therapeutics in light of the phenotype of $CFTR^{-/-}$ sheep. According to Xu *et al*. [25], CFTR expression patterns in rabbits are very similar to those in humans. The bioelectric characteristics of the nasal and tracheal epithelia in CF rabbits showed anomalies similar to those found in human CF. Schwank *et al*. [26] investigated cystic fibrosis treatment using CRISPR/Cas9. They were able to successfully repair the most typical CF-

causing mutation in intestinal organoids using mature intestinal stem cells taken from two CF patients.

About 3500 to 5000 males worldwide are affected by the fatal X-linked recessive neuromuscular disease known as Duchenne muscular dystrophy (DMD) [27]. Zhang *et al*. [28] introduced the dual AAV system into a mouse model of DMD along with Cas9 nuclease and CRISPR single guide RNAs packed into single-stranded AAV (ssAAV) and self-complementary AAV (scAAV), respectively. At least 20 times less scAAV was needed than ssAAV to achieve effective genome editing. In mice receiving systemic therapy, dystrophin expression was restored, and muscular contractility was enhanced [28]. In addition, Min *et al*. [29] demonstrated a quick and effective method for correcting exon 44 reduction abnormalities by CRISPR/Cas9 in cardiomyocytes grown from physician-induced pluripotent stem cells as well as in a novel mouse model with the same deletion mutant. They also showed the significance of the doses of these gene-editing components for successful gene correction in vivo using AAV9 expressing Cas9 and single guide RNAs. In a mouse model of DMD, Tabebordbar *et al*. [30] restored dystrophin production by deleting the exon bearing the initial mutation using adeno-associated virus (AAV) delivery of CRISPR/Cas9 endonuclease. As a result, a functional shortened protein was produced. A partial recovery of muscle functional deficits was observed in treated mice. They showed that the dystrophin gene was altered in muscular stem cells that replaced matured muscle tissue. To ensure that any pharmacological effects of CRISPR/Cas9 did not diminish over time, this was crucial [31, 32].

A set of inherited blood illnesses and diseases that largely affect red blood cells are collectively referred to as hemoglobinopathies in medical science. Without any use of HDR enhancers, Lamsfus-Calle *et al*. [33] achieved significant rates of gene addition and correction in hematopoietic stem and parent cells from gutsy donors. These methods were also tested on individuals with heterozygous and homozygous thalassemia, resulting in a considerable rise in HbA and proving the study's universal therapeutic promise for the treatment of hemoglobinopathies [33]. A phase one experiment in the USA, using the CRISPR-Cas9 technology, successfully treated the first individual with sickle cell disease, according to a report from CRISPR Therapeutics in 2019 [34]. Canver *et al*. [35] demonstrated that CRISPR/Cas9 *BCL11A* promoter disruption could produce fetal hemoglobin in both human erythroblast cells and mice.

3.1.2 Treatment of HIV/AIDS

HIV is a destructive virus for human life. CRISPR/Cas9 can play a significant role in treating this disease. Currently, no permanent treatment exists for this disease. According to Xiao *et al*. [6], CRISPR/Cas9 can be utilized to target cellular cofactors or the HIV-1 genome to decrease HIV-1 infection and eradicate the provirus. It can also be used to promote the transcriptional activation of latent viruses to eradicate them from latent viral reservoirs. Hu *et al*. [60] showed that the HIV-1 genome can be inactivated by the action of the CRISPR/Cas9 system. The researchers used a greater genomic sequence sgRNA library in a CRISPR/Cas9 constitutively active screen to find the biological components that prevent human $CD4^+$ T cells from becoming infected with HIV-1 [36]. Many scientists all over the world are trying their hardest to find the accurate genome sequence to treat HIV by using the CRISPR/Cas9 tool.

3.2 Use in plant science

Since CRISPR/Cas9 gained popularity as a gene-editing technique, various studies have been conducted to employ this tool to increase crop yield and some of these have achieved substantial success. CRISPR/Cas9 has quickly been used for nearly all-important crops, including wheat, rice, and cotton [37]. These days, this technology is used all over the world to increase crop yield,

development, and growth as well as the plant's ability to respond to various stresses, including salinity and drought.

3.2.1 Contribution of CRISPR/Cas9 in plants to biotic stress

Attacks by pests and pathogens are a major issue for crop cultivation. Complete yield loss happens as a result of the extensive harm that pests and pathogens cause to crops [37]. Numerous plant species, including wheat and tomatoes, are susceptible to the widespread illness known as powdery mildew, which is caused by fungi [38]. Powdery mildew disease is created by *MILDEW-RESISTANCE LOCUS (MLO)* genes in barley [39]. Shortly after the CRISPR/Cas9 technique was applied in plant research, Wang *et al*. [40] confirmed that the deletion of three MLO genes by transcription activator-like effector nuclease (TALEN) in wheat resulted in wheat resistance to powdery mildew (PM) disease. After that, much research was done in this field. Martínez *et al*. [41] developed the *PMR4* gene in tomato plants, which were resistant to PM. For the *SlMlo1* gene, Pramanik *et al*. [42] produced knockout lines that completely exhibited resistance to powdery mildew. The *VvPR4b* and *VvMLO3* genes were edited through CRISPR/Cas9, increasing the grapevine's (*Vitis vinifera L.*) resistance to PM [43, 44].

Fusarium wilt is another dangerous fungal disease caused by *Fusarium oxysporum f.sp. niveum* (FON), and can cause 30-80% yield loss in watermelon [45]. In the past, there was no FON-resistant variety [46]. Hammes [47] were successful in deleting the *Clpsk1* gene, which encodes the precursor of phytosulfokine (PSK), a desulfated pentapeptide plant hormone that controls resistance in plants. This greatly improved the watermelon plant's resilience to Fusarium wilt disease [46].

The CRISPR/Cas9 system was used to create turnip mosaic virus resistance in Arabidopsis by targeting the *eIF(iso)4E* gene [48]. Rice became resistant to *Magnaporthe oryzae* and *Xanthomonas oryzae pv. oryzae* after targeting with the *OsERF922* and *Os8n3* genes, respectively [49, 50]. Tomato, citrus, and orange became resistant to bacterial disease by knocking out the *SlJAZ2, CsLOB1,* and *CsWRKY22* genes, respectively [51-53]. The CRISPR/Cas9 tool was used to knock out the *eif4e* gene, which produces virus resistance to cucumber vein yellowing virus, papaya ring spot mosaic virus W, and zucchini yellow mosaic virus [54]. Cotton became resistant to the fungal disease *Verticillium dahliae* after knocking out the *14-3-3* gene with the application of CRISPR/Cas9 [55].

3.2.2 Contribution of CRISPR/Cas9 in plants to abiotic stress

Abiotic stress is getting worse for crop production as a result of global warming. The most frequent abiotic stresses are drought, salinity, waterlogging, and extremely high or low temperatures [34]. By modifying the genome sequence using the CRISPR/Cas9 technology, plants can be made to cope with these types of stress far more effectively (Figure 4).

The *AGROS8* gene increased the tolerance ability of maize to drought conditions by the use of CRISPR/Cas9 technology [56]. *SlAGL6* (a R gene) was knocked out using CRISPR/Cas9 technology, which improved tomato plant growth and fruit development even when the plansts were exposed to heat stress [57]. Plants can tolerate drought stress by deletion of *dpa4-sod7-aitr256* with the help of the CRISPR/Cas9 tool [58]. The CRISPR/Cas9 editing tool was used to produce salinity and osmotic stress-tolerant tomato plants by knockdown and knockout of the *ARF4* gene [59]. In another study, tomato plant ability to withstand drought was improved by a *SlLBD40* gene mutation [60].

Rice can tolerate salinity stress by deletion of G-genes (*gs3* and *dep1*) [61]. Rice was more resistant to alkaline stress when the *ppa6* gene was knocked out [62]. Drought and salt tolerance rice were developed by CRISPR/Cas9 system, which knocked out the *OsDST* gene [15]. Enhancing responses to abscisic acid and drought stress in rice were achieved using CRISPR/Cas9 to target *OsERA1 gene* mutation [63]. The majority of research demonstrated improved plant tolerance to

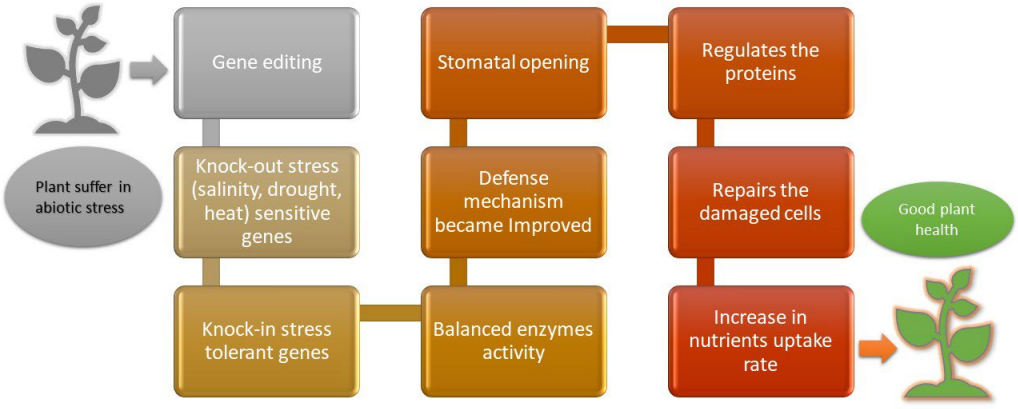

Figure 4. Periodic process of plant growth and development by CRISPR/Cas9 technology

abiotic stressors in CRISPR-edited plants, while some studies also demonstrated the opposite outcome. For instance, tomato plant ability to withstand drought stress was decreased by the CRISPR/Cas9-mediated deletion of gene 1 (*npr1*) [64].

4. Limitations

Although its use in plant science is very efficient and effective, there are some limitations on the use of the CRISPR/Cas9 system in medical science. In the USA and many other nations, germline cell and embryonic genome editing are now totally prohibited due to ethical and indemnity issues [65]. Since it is a new technology, it takes a little time to get through all levels.

The Protospacer adjacent motif (PAM) is the most significant part of the CRISPR/Cas9 system, which provides a specification that guides the editing site [66]. PAM sequences the necessary genes that make the CRISPR/Cas9 system's work more potent and efficient. A big limitation is the lack of a proper PAM sequence. A dependable delivery strategy is lacking for the CRISPR/Cas9 technology. The CRISPR/Cas system's delivery strategy is crucial. It impacts not only the effectiveness of the transformation but also the off-target effect and subsequent regulation goals. For CRISPR/Cas applications, the off-target effect is a major concern [67].

5. Future Work

The expeditious progress of CRISPR/Cas9 has boundless potential for both fundamental and practical research. It is now one of the swiftly arising influential biotechnological techniques which can help in intercepting significant biological and agricultural problems. Genome editing based on CRISPR/Cas9 will also emerge as a key breeding technique for agricultural and human development. No technology has been created and deployed in biological and medicinal research as swiftly over the last eight years as CRISPR/Cas9.

With the goal of eventually deploying the technique to consistently treat diseases in humans, much research is still concentrated on its usage in isolated human cells or animal models. There is a vast area of research to prevent the "off-target" effects of the CRISPR/Cas9 system, which

involves the editing of a gene other than the one that was meant to be modified [68]. Better DNA targeting is required for the proper use of CRISPR/Cas9 tools.

The guide RNA typically consists of a particular 20-base sequence. The repeating unit in the gene that will be altered is complementary to these bases. The guide RNA can bind even if only some of the 20 bases are the same. This presents a dilemma since a sequence that contains, for instance, 19 of the 20 complimentary nucleotides can reside in a completely different location in the genome. According to this information, the gRNA may bind in addition to or instead of the target sequence.

The Cas9 enzyme then makes a cut at the incorrect spot, leading to the introduction of a mutation instead. Even while this mutation might not have any impact on the person, it might have an impact on a critical gene or another significant portion of the genome. The accuracy of the CRISPR-binding Cas9 and cutting is a major concern for scientists. There are two ways to accomplish this: the creation of better, more targeted gRNAs using our understanding of the genome's DNA sequence and the 'off-target' behavior of various Cas9-gRNA complexes. The Cas9 enzyme is being used to split the single strand of the target DNA. Two Cas9 enzymes and two guide RNAs need to be present simultaneously to function. As a result, there is a lower chance that the cut will be made incorrectly.

Most of the genome editing modifications are only made to somatic cells, and some of the specific tissues are being affected by these alterations. They cannot be passed on from one generation to the next. However, modifications to an embryo's, sperm's, or egg cells' genes may be passed on to the following generations. Genome editing of germ cells and developing embryos raises a number of ethical issues, such as whether it would be acceptable to utilize this technology to improve typical human qualities (such as height, color, or intelligence).

6. Conclusions

CRISPR/Cas9 is a widely used genome editing technology that can make it possible to get rid of many grim diseases in humans. At present, with the help of this method, it is possible to get high-yielding varieties of crops. The CRISPR/Cas9 system has been used to improve crop growth by displacing certain genes, thus making plants better able to survive in adverse environments. Other crops have become resistant to pathogens through the action of the CRISPR/Cas9 gene editing tool. A current goal of CRISPR/Cas9 research is to create a more effective and reliable system, especially a CRISPR/Cas9 reagent delivery method that generates fewer off-target effects.

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