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Application of Gene Editing Technology in Agricultural Germplasm Resources

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Abstract: Gene editing technology can be used to precisely manipulate specific sites in the genome to achieve DNA fragment deletion, DNA insertion, or single base mutations. This technology has helped overcome the challenges in traditional agricultural breeding and has been widely used to improve the economic traits of animals and plants, which can create a variety of new germplasms for breeding. The CRISPR/Cas9 technology has become the mainstream technology in current applications because of its unique advantages such as ease of handling, high efficiency, multi-targeting ability, and versatility. Therefore, it can potentially bring about revolutionary changes in agriculture. To improve the future of agricultural breeding in the seed industry of China, it is necessary to improve the efficiency and accuracy of this technology and to establish laws and regulations related to it.
Keywords: gene editing technology; agricultural breeding; agricultural germplasm innovation

1 Introduction

1.1 Definition

Gene editing is a precise genetic engineering technique used to achieve DNA fragment knockout, DNA insertion, or single base changes at specific sites within the genome. Gene editing involves two main strategies. The first is to find the exact location on the genome in order to target the specific DNA fragments to be edited. The second is to perform DNA editing using nucleases. After the genomic DNA is broken up by nucleases, a base is inserted or deleted resulting in the inactivation of the functional gene via non-homologous end joining (NHEJ). DNA can also be repaired by homology-directed repair (HDR), which can lead to DNA fragment insertion or base mutation. Second-generation gene editing technology, which involves zinc-finger nucleases (ZFNs) and transcriptional activator-like effector nucleases (TALEN), has greatly improved the efficiency of gene editing. In 2013, the development of the CRISPR/Cas9 system represented a breakthrough in the third generation of gene editing technology. With unique advantages such as ease of handling, low cost, high efficiency, and the ability to modify multiple targets, the CRISPR/Cas9 system has been used successfully to produce porcine reproductive and respiratory syndrome virus (PRRSV)-resistant pigs and hornless cows, effectively overcoming the issues associated with conventional breeding. Thus, it has quickly become a mainstream gene editing technology. With continuous improvement in the field of gene editing technology and through its widespread application in animals and plants, it can bring about revolutionary changes in the field of agriculture in the near future.

1.2 Technical characteristics

The characteristics of CRISPR/Cas9 gene editing technology can be summarized as follows: (1) Ease of handling and low cost: The gene editing system involves the construction of only a Cas9 gene and sgRNA expression plasmid

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and functions via the enzymatic cleavage activity of Cas9 protein and the ability of the sgRNA to guide the Cas9 protein to the target sites. (2) Higher editing efficiency: The cleavage efficiency of the targeted double-stranded DNA is over 50%, and can even go up to 90%. In addition, the homologous recombination efficiency mediated by this technology is over 40%, which is much higher than that achieved with previous methods. (3) Broad targeting coverage and high specificity: Using the PAM sequence (NGG) as a recognition sequence, this technology can be used to edit all the genes of the target genome (there is one PAM sequence every 8 bp in the human genome). Moreover, by matching the restriction seed sequence of the sgRNA (8-12 bases from the PAM sequence) completely with the target DNA, the targeting specificity can be further enhanced. (4) Editing multiple genes simultaneously: Cas9 protein can form a complex with several different sgRNAs at the same time, allowing multiple genes to be edited simultaneously. (5) Versatility: With practically no restrictions on the types of genes, cells, or species that can be edited, this system is suitable for modifying the genome of agricultural animals, plants, or microorganisms (viruses, bacteria).

2 Development and application status of gene editing technology

2.1 The development of gene editing technology

In the 1980s, Mario R. Capecchi et al. achieved a milestone by developing gene targeting technology that allowed accurate editing of animal cell genomes [1]. With gradual advances in the research associated with it, there have been continuous breakthroughs in this technology and various technical systems have been established. Among them, the ZFN technology was the earliest artificial endonuclease-mediated genome editing technology to be established and it has been successfully applied to many animals and plants such as corn, mice, humans, fruit flies, zebrafish, cattle, and pigs [2]. Although this technology has a high editing efficiency, its application is difficult and expensive, which limits its promotion and application. Around the same time, another gene editing system, TALEN technology, was developed, which is relatively simple to execute and can also produce double-strand breaks at specific target DNA sequences to achieve accurate gene editing [3] with an average editing efficiency of over 40%. It has been applied to yeast, fruit flies, zebrafish, Xenopus, mice, rats, rice, pigs, cattle, sheep, and other organisms. There has been significant improvement in the editing efficiency from the first generation of gene targeting to the second-generation ZFN and TALEN technologies. In 2012, American scientists first confirmed that the CRISPR/Cas9 system can target DNA sequences precisely and can be used for gene editing of the cellular genomes of animals [4−6]. This marked a breakthrough in targeted genome modification in animals. Since then, the CRISPR/Cas9 gene editing technology has been gradually applied to other organisms such as animals, plants, and microorganisms, and has become an efficient and universal mainstream gene editing technology. Through the binding of mutant Cas9 with cytosine deaminase, uracil glycosidase inhibitory protein, and adenosine deaminase, the base editor was developed to make a single base conversion possible [7,8].

2.2 Fields that have applied gene editing technology

2.2.1 Overcoming the challenges in the genetic improvement of animals and cultivation of new breeds

Traditional animal breeding can be time consuming and the targeting of traits that need to be improved is uncertain. It is also difficult to obtain a combination of desirable traits. Gene editing technology not only helps to overcome the genetic barriers of traditional breeding but also allows the precise alteration of specific traits. This technology was an advancement over existing methods for improving animal genetics and breeding efficiency. The development of gene editing technology has already been made a priority in developed countries as well as emerging economies in order to be up-to-date about the future advances in animal breeding.

(1) Improved production performance

Breeders have found that myostatin (MSTN) is a negative regulator of muscle growth. The Belgian blue and Piedmont cattle have a naturally mutated MSTN gene and have high muscle mass, which is 18% to 20% higher than that of other cattle. Therefore, to expedite the progress of improving the meat quality during animal breeding, pigs, cattle, and sheep that originally did not have any MSTN gene function were created using gene editing technology. Consequently, the muscle growth rate and lean mass was significantly increased resulting in the classic “double muscle hip” feature in these animals [9−11]. In addition, the horn phenotype of cattle is not conducive for feeding management, and conventional breeding to produce hornless cattle requires at least 20 years and may result in decreased milk production. To address this issue, American scientists edited the horn alleles of the genome of the Holstein cattle and replaced them with the hornless P_c gene to create hornless cattle [12], resulting in reduction in

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animal injuries and improved milk production. Additionally, the editing of the FGF5 gene increased the amount and the fineness of the wool produced by sheep.

(2) Improving product quality

β-lactoglobulin is an intrinsic protein found in the milk from goats and cattle. It is one of the main allergens that causes milk allergies in infants (an infant's digestive system is not yet fully developed and absorbs the *β*-lactoglobulin as a whole, and the absorbed *β*-lactoglobulin is thus identified as a pathogen by the immune system). However, traditional breeding methods cannot be used to remove this allergen. In 2011, scientists from China first knocked out the *β*-lactoglobulin gene in dairy cows using gene editing technology, creating cows that can produce milk without *β*-lactoglobulin, and this milk can be used to produce dairy products suitable for infants [13]. This study inspired the creation of new breed of dairy goats with the edited *β*-lactoglobulin gene, which is also present in goat milk. Hence, people who are allergic to dairy milk products can now consume them safely due to the lack of *β*-lactoglobulin.

(3) Increasing disease resistance

Severe infectious diseases in animals not only threaten human health but also result in huge economic losses for the animal husbandry industry. While such diseases have always been an industrial problem that has challenged breeding experts and epidemiologists, gene editing technology provides effective solutions to overcome this problem. For example, spongiform encephalopathy is an infectious disease caused by presence of the PRNP gene in cattle, sheep, and humans. Chinese researchers have used gene editing technology to knock out the PRNP gene and obtain new breeding material that can provide protection against mad cow disease in sheep and goats [14−16]. Similarly, the anti-tuberculosis mouse gene Ipr1 was introduced into the cow genome, using gene editing to breed cows resistant to tuberculosis [17]. Using gene editing technology, American scientists introduced a loss of function in the gene for the PRRSV binding receptor CD163 to obtain pigs that are resistant to PRRSV, thus preventing one of the most damaging infectious diseases in the porcine industry [18,19].

2.2.2 Overcomming the issues slowing yield increase, quality improvement, disease resistance, and stress tolerance in crop breeding.

(1) Yield increase and quality improvement

The yield of a crop is influenced by many minor polygenes. The progress made by traditional breeding methods in improving the genetics has been slow. However, gene editing techniques have been used to create mutant varieties of rice with enhanced grain number, dense erect panicles, larger grain size, and fewer tillers by knocking out the genes that negatively regulate the yield such as, Gn1a, DEP1, GS3, and IPA1 [20−22]. Moreover, editing the negative regulator gene, TaGASR7, which controls the grain length and weight of wheat, using CRISPR/Cas9 technology has also significantly increased the weight of the wheat grain. Amylose content is a property that affects the quality of rice. Targeted modification of the amylose synthase gene, OsWaxy, using CRISPR/Cas9 technology decreased the amylose content in the mutants from 14.6% to 2.6% [23], which improved the glutinous quality of rice. This technology has also been used to significantly increase the lysine content in O2 mutant corn by knocking out the O2 gene, which affects the lysine content in maize. Similarly, editing the genes fl2, opaque7, opaque6, and De-30 changed the amino acid composition of the endosperm and increased the lysine content [24]. Therefore, gene editing technology has become an important tool for the rapid and specific improvement of crop yield and quality.

(2) Improvement of stress tolerance and disease resistance

Rice blast is one of the most destructive diseases affecting rice. The OsERF922 gene is a negative regulator of rice blast resistance, and when it was knocked out, the T2 homozygous mutant exhibited significantly increased resistance to *Magnaporthe oryzae* at the seedling and the tiller stage [25]. American scientists edited the Sweet14 gene to obtain rice resistant to bacterial blight [26]. For the first time, in China, the wheat MLO gene was edited, and a variety of wheat with broad-spectrum resistance to powdery mildew were obtained [26]. In maize, the gene, ARGOS8, is a negative regulator of ethylene responses. A study showed that the over-expression of ARGOS8 under drought conditions significantly increased the yield compared to the wild type. The expression of ARGOS8 was also significantly enhanced by gene editing and the yield of the mutant was much higher than the wild type under drought conditions [27].

2.2.3 Overcoming the challenges in animal vaccine development and pathogenesis research

The first candidate vaccine produced using gene editing was created in China by editing the pseudorabies virus using the CRISPR/Cas9 system and Cre/Lox system. However, this approach is not suitable for all pathogen species, especially in case of pathogenic microorganisms such as *Mycobacterium tuberculosis*. However, the new

CRISPR/Cas gene editing system, which is based on the Type III CRISPR/Cas 10 system, can be used for the gene editing of different pathogenic microorganisms and for producing gene edited vaccines such as those against PRV, African swine fever virus (ASFV), and *M. tuberculosis*. Furthermore, a focused CRISPR/Cas-based library or whole-genome CRISPR interference (CRISPRi) screening platform has been used to screen viral receptors genes in humans, mice, as well as other animals. It has also been used to identify the key factors associated with viral replication. For example, the innate immune receptor for the bacterial ADP-heptose was identified using the whole-genome CRISPRi screening platform [28].

3 Industrialization prospects for gene editing technology

3.1 High demand for the industrialization of gene editing technology

CRISPR/CAS9 gene editing is a revolutionary technology that has not only caused a sensation in academia and industry but also has attracted the attention of investors. With its gradual penetration into agricultural breeding and other fields, a series of breakthroughs have been made, such as the production of anti-PRRSV pigs, hornless cattle, and phosphorus-efficient corn. With these developments, gene editing technology is gradually moving towards industrialization. Scientists from the University of Missouri successfully obtained CD163 knockout pigs that are completely resistant to PRRSV through gene editing in 2014. The world famous porcine breeding company, Genus PLC has cooperated with the University of Missouri to develop a breeding system for this new anti- PRRSV porcine breed. Moreover, in 2015, they announced that this anti-PRRSV pig would be made available to pork producers within the next 5 years. This company has successively obtained the intellectual property rights to the CD163 gene and molecular scissors, and they currently have a grandparent herd population of anti-PRRSV pigs [29]. Compared to animal research, the development and applications of gene editing products in plants are relatively more sophisticated, as they have been used for commercial production. In 2012, the US government approved the first ever phosphorus-efficient maize created using targeted genome modification, which can be applied for field evaluation directly and it is considered as a conventional variety. The great economic value of gene editing technology for imparting disease resistance, herbicide resistance, and quality and yield improvements in plants has driven industrial research and development investments by the Bayer, Syngenta, Monsanto, and DuPont-Pioneer companies. In addition, this technology has also greatly inspired the creation of new crop varieties.

3.2 Industrialized market size forecasts for gene editing technology

With technical developments, the advantages of gene editing technology have been increasingly highlighted and gradually transformed into industrial assets. Currently, the technology has widespread applications in the fields of animal and plant breeding. An estimation by the Kalorama Information Company shows that the market size of gene editing and relevant supply is expected to surpass 5 billion US dollars by 2025. In China, for instance, in 2017, there were 403.5 million living pigs, 100.85 million living beef cattle, 13.766 million living dairy cattle, and 303.14 million living mutton sheep, making China one of the major practitioners of animal husbandry. However, with growth in population, the demand for quality livestock products has increased and hence, the quantity of livestock will continue to increase dramatically. Even if 10% to 20% of the animals will be replaced by gene-edited animals, the market size for gene-edited animals in China will still be considerably large. Similarly, the crop market will also be massive and will have more significant economic benefits.

4 Suggestions for the industrial development of gene editing technology

Even though the industrialization of gene editing technology is closely related to the market demand and has attractive prospects, there are still several bottlenecks remaining. First, there are a few inherent technical problems in the CRISPR/Cas9 system. Currently, this technology has a relatively low gene editing efficiency and its target specificity also needs to be improved. Second, there are technical barriers in the industrialization of CRISPR/Cas9 technology. CRISPR/Cas9 technology was originally developed by American scientists and the ownership of the intellectual property belongs to the United States. Therefore, the application of this technology in agricultural breeding will involve intellectual property issues. China needs to focus on the relevant basic research and on developing new gene editing technologies to avoid patent right infringement. Third, there are law and regulation issues associated with the industrialization of gene editing technology. Whether gene editing technology can be industrialized or not mainly depends on the completeness of the corresponding laws and regulations. Europe and the United States are leading the world in these relevant laws and legislation, which provides legal protection for the

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industrialization and application of gene editing technology. Thus, to promote the industrialization of gene editing technology, China needs to coordinate work in the following aspects.

4.1 Planning the layout of the science and technology programs

China needs to enhance the top-level design and overall layout of the technical programs, address the issues associated with the application and promotion of gene editing in the field of agriculture, carry out fundamental and applied research that are both cutting-edge as well as original, address the major strategic needs of its economic and social development in the coming 20 years, and launch and implement gene editing science and technology programs in the field of agriculture.

4.2 Vigorous promotion of the co-operation between industries, universities, and research centers

Gene editing is a knowledge-intensive technology, and its industrial development can be possible through the cooperation between industries, universities, and research centers. With the help of agricultural science and technology innovation programs, China needs to cultivate and gather leading talent and excellent teams that can support industrial development and innovation to provide intellectual support for the industrialization of gene editing technology. China should also encourage universities and research institutions to engage in agricultural, scientific, and technological collaborations and the commercialization of research findings in various forms; increase the venture investment with business and social capitals to solve common funding shortage issues faced by production, teaching, and research; and promote the practical applications of gene editing technology in the field of industrial agriculture.

4.3 Introduction of social capital investment

As an effective new technology, gene editing will inevitably trigger a revolutionary change in the field of agriculture and bring immeasurable economic benefits. In addition to increasing the financial input into the R&D for agricultural gene editing technology, the Chinese government should also formulate relevant preferential policies that will encourage and guide the investment of social capital to accelerate the industrialization and application of gene editing in the field of agriculture.

4.4 Formulation and improvement of relevant laws and regulations

Gene editing is a novel technology for the genetic improvement of animals and plants and it is fundamentally different from transgenic breeding. Corresponding laws and regulations have been established in developed countries. However, in China, no laws or regulations have been formulated as yet for the use of gene editing technology. Thus, the Chinese government should pay close attention to the technological development in this area and approve relevant regulations and rules for their implementation in developing agricultural gene editing products, thus paving the way for the industrialization of gene editing technology in the field of agriculture.

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