Review Article

CRISPR Technology in Agriculture: Applications, Challenges, and Bioethical Considerations

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Abstract

The growing global food demand in agriculture, mitigating the effects of climate change, and creating sustainable production systems have necessitated the application of modern biotechnology. In recent years, CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) technology has been in the spotlight as one of the most promising approaches in the field of gene editing. This technology allows for highly precise changes in plant and animal genomes, thereby increasing productivity, quality, and potential uses in animal husbandry. In addition, bioethical issues arising from the application of the technology, possible impacts on ecological balance, protection of biodiversity, consumer acceptance, animal welfare, and current problems related to the regulation of genetically modified organisms are discussed.

Keywords: CRISPR, gene editing, agriculture, plant breeding, productivity, bioethics, animal welfare, biosafety

1. Introduction

One of the most significant steps in the application of the CRISPR/Cas system in plants is the efficient delivery of editing reagents into plant cells, resulting in the occurrence of genome editing events. The main editing reagents used for this purpose include DNA, RNA, and ribonucleoprotein complexes (RNP). These reagents can be introduced into plant cells by various methods. The most widely used methods are protoplast transfection, Agrobacterium-mediated T-DNA transformation, and particle bombardment (biolistic method). Protoplast transfection is mainly used for transient gene expression. In contrast, Agrobacterium-mediated transformation and particle bombardment are the primary and most widely used delivery methods for generating edited plants [1].

The most commonly used genetic material for plant genome editing is CRISPR/Cas DNA. Once introduced into a cell, the DNA cassette expressing the Cas protein and sgRNA can either be degraded and lost, or it can be randomly integrated into the plant genome. In the traditional DNA transformation approach, CRISPR/Cas DNA is introduced into recipient cells by Agrobacterium-mediated transformation or particle bombardment. This DNA is then integrated into the plant genome through the selection of marker genes and expressed, leading to genome editing. This strategy has been used in many types of plant genome editing. However, the permanent placement of CRISPR constructs and marker genes in the genome can have certain negative effects. In particular, this situation can lead to an increase in off-target changes, which may ultimately limit the commercial applications of the technology [1].

To avoid these problems, it is possible to obtain transgenic-free derivatives, which are mainly obtained through genetic segregation. As a result of self-pollination or crossing of plants, non-transgenic mutants are obtained by selecting progeny that do not carry the CRISPR construct. For example, Gao and colleagues have used a fluorescent marker cassette to monitor the presence of a CRISPR/Cas9 construct. Another interesting approach is to use suicide genes called CMS2 and BARNASE. These genes facilitate the selection of non-transgenic plants by eliminating transgene-bearing pollen and embryos produced in the T0 plant. However, the genetic segregation method is not suitable for vegetatively propagated plants. For example, this method cannot be used in plants such as potatoes, cassava (Manihot esculenta), and bananas (Musa species). In addition, there is a

possibility that certain fragments of the DNA construct used may integrate into unknown regions of the genome [1].

Transient gene expression-based delivery of CRISPR reagents is considered an alternative approach to achieve transgene-free genome editing. This method does not involve classical selection steps using herbicides or antibiotics, and as a result, some regenerated plants are edited without any integration of foreign DNA into the genome. This method was first applied to wheat plants. In that study, the CRISPR/Cas9 plasmid was introduced into immature wheat embryos via particle bombardment, and the plants were then regenerated without the application of selection pressure. This approach reduced the regeneration time by approximately 3–4 weeks through tissue culture. The mutation rate was comparable to that of traditional DNA integration methods with selection. One of the necessary results is that 86.8 percent of T0 mutants did not contain transgenes [1].

In addition, DNA base editors have also been introduced into plants by transient delivery. These editors include cytidine base editors and adenine base editors, and have been used to achieve non-transgenic nucleotide substitutions. In addition to biolistic delivery, non-transgenic editing has also been achieved by other methods. For example, genome editing has been successfully achieved through protoplast transformation in tetraploid potato and tobacco protoplasts, as well as transient expression of the CRISPR/Cas system in tobacco via Agrobacterium. These approaches demonstrate that CRISPR technology has the potential to enable the development of safer and more efficient methods for plant genome editing [1].

The aim of this review is to evaluate the current and potential applications of CRISPR technology in agriculture, to explore its contribution to improving crop and livestock production, and to discuss bioethical and biosafety issues related to the application of the technology.

2. Improving Plant Quality and Productivity

CRISPR/Cas9 gene editing technology has become one of the most important tools in agriculture in recent years to improve the quality and productivity of plants. As a result of the development of this technology and the more accurate study of plant genome data, researchers have been able to change specific genes in plants with high precision. Compared to traditional breeding methods, CRISPR/Cas9 allows for faster and more effective results [2].

In modern times, CRISPR/Cas9 technology has opened up new possibilities in this field. Through this method, genes that have important functions in plants can be precisely edited. As a result, plant productivity is increased, quality is improved, and the selection process is carried out more efficiently. With the help of CRISPR/Cas9 technology, it is possible to improve the external and internal quality indicators of plants. External quality characteristics include the size, color, external structure, texture, and appearance of fruits and seeds. Internal quality characteristics include the amount of protein, carbohydrates, starch, fats, vitamins, and various bioactive substances [3]. For this reason, CRISPR/Cas9 technology is considered by many breeders as one of the most promising and widely applied strategies [2].

The studies conducted examined the achievements achieved as a result of the application of CRISPR/Cas9 technology in various plant species and analyzed the current status of this technology in improving the quality of plants. In addition, the shortcomings and difficulties encountered during the application of this method were also considered, and ideas and directions for future development prospects were put forward [2].

3. Regulation of Gene Expression and Editing of the Plant Genome

Recent studies show that most plant breeding work using CRISPR/Cas technology has focused primarily on the coding parts of genes. In contrast, relatively little research has been done on the regulatory regions that regulate gene expression and control gene activity. However, these regulatory regions are important elements that determine when and at what level genes are expressed and can have significant effects on plant traits [4].

Engineered nucleases were incorporated into a non-specific nuclease domain fused to a sequence-specific DNA binding domain. Such fused nucleases form a structure that can precisely cleave the target gene, and the breaks are repaired by NHEJ or HDR. This structure is called “genome editing”. Before the advent of CRISPR-Cas9 technology, genome editing used first-generation genome editing tools such as meganucleases, zinc finger nucleases (ZFNs), and TALENs [5]. However, these systems required complex, time-consuming, and tedious procedures to achieve high target specificity. CRISPR-Cas9, on the other hand, has created a new era

in genome editing due to its easier design, high precision, and low cost. Therefore, genome editing is widely used in modern plant breeding, plant biology research, and also in animal systems [6]. Then, the importance of regulatory regions involved in the regulation of gene expression in plants was explained. In particular, in recent years, the application of mutations created in these regulatory regions using CRISPR/Cas technology in plant breeding and the results obtained have been in the spotlight [4].

In addition, promising directions for how genome editing technologies can be used in plant breeding in the future are presented. Overall, this approach may open up new research directions for plant improvement using genome editing methods and allow for the development of more effective breeding strategies [4].

4. Ethical Issues

4.1. Bioethical Issues

CRISPR-Cas9 is widely recognised as one of the most significant discoveries of the 21st century by the scientific community and associated industries. CRISPR-Cas9 technology is a complex field that raises ethical, social, and legal controversies beyond gene editing. The technology has rapidly gained acceptance in animals and the environment, but has also recently raised difficult questions, applications, concerns, and bioethical research in humans [7]. However, the rapid rise of CRISPR-Cas9 has led to new bioethical, social, and legal issues in medicine [8], agriculture, livestock, and the environment [7]. CRISPR-Cas9 is used to genetically modify human germ cells and embryos. This is called germline genome editing. Germline genome editing raises a series of bioethical issues, such as the occurrence of unwanted changes in the genome, who and how to obtain informed consent, and the breeding of the human species (eugenics). Also, the bioethical issues that CRISPR-Cas9 technology may raise in the environment, agriculture, and animal husbandry should not be forgotten. To this end, legislation should be developed worldwide, taking into account the views of both life and social scientists, policymakers, and all other stakeholders in the sectors, to ensure that CRISPR-Cas9 can be used safely in all areas and that potential problems are addressed, and CRISPR-Cas9 applications should be carried out in accordance with this legislation. However, these control measures should not restrict scientific freedom [7].

4.2. Ecological Imbalance

Nontarget effects should be thoroughly investigated in studies that employ RNA-targeted gene editing methods based on CRISPR-Cas9. Possible off-target mutations will continue to exist in each generation due to an ongoing process of gene transfer in a population. Furthermore, the frequency and impact of mutations may increase as generations advance. The potential for genes to transfer to other species in the environment is a further concern. Negative characteristics may be transferred to related organisms if regulated sequences are transferred to other species. The distribution of the properties of the entrained genes among the populations can make control very difficult [7].

4.3. Regulations for Consumers

It is extremely challenging to recognise and control genetically modified organisms (GMOs) once they leave the lab due to the use of CRISPR-Cas9 to achieve the required genetic alterations. Thus, regulatory agencies like the European Medicines Agency (EMA), the US Food and Drug Administration (FDA), and others should consider whether any GMOs are safe for consumers. However, it is not known exactly how to evaluate the possibilities of a growing market with CRISPR-Cas9 [7]. There are high prospects for making and taking up CRISPR-Cas9 technology for commercialization in many fields, including health, food, and biologics production. It is relevant for the commercialization of CRISPR technology given that it continues to experience regulatory, ethical, and market forces that are key to its future growth [9].

Patenting is one of the CRISPR-Cas9 issues that affects all of humanity. As is well known, certain human gene sequences for therapeutic applications as well as transgenic organisms used in industry have been patented. In the future, patent-related problems in many biotechnology fields will keep increasing as technologies like CRISPR continue to develop. Several cases of patenting still exist today. The patent rights case between Zhang, Doudna, and Charpentier about the therapeutic application of CRISPR-Cas9 in human cells is the most well-known. In the case concluded on 2 December 2016, it was decided to grant the patent to Caribou Biosciences, of which Doudna was the founder [7].

4.4. Generation of Chimeric Animals for Organ Transplantation

Organ transplantation is the process of replacing an organ that is no longer functioning in a person's body with a healthy organ from a living donor or dead body. The primary goal is to save the patient's life, who is at risk of dying from organ failure, as well as to extend their lifetime and improve their quality of life. The development of chimeric animals may prevent patients from spending precious time waiting for an appropriate donor [7]. Organ transplantation is the most promising cure for end–stage organ disease. Human-animal chimeras provide the ability to produce human organs in another species using autologous stem cells (e.g., induced pluripotent stem cells (iPSCs) or adult stem cells), which would be patient-specific and immune-matched for transplantation. However, organ shortage and substantial risk of rejection have limited the development of organ transplantation. Therefore, finding more resources for organ transplantation is urgently needed [10].

Chimaeras include human neurons and germ cells, which raises bioethical concerns. The two fundamental concerns can be summarised as defining the order of nature and the moral disorders induced by how the organism is handled, depending on whether it is classified as human or animal. This method has received a lot of attention because of the potential use of interspecies chimaeras in fundamental and translational research. Some people believe that chimeric embryos will have an impact on human dignity and identity because they have the ability to create organisms from human cells and tissues. Others argue that chimaera organisms containing human cells cannot transform into humans and hence do not threaten human dignity. They also argue that the human-like features imparted to chimeras will neither affect the biological environment nor the moral status of animals and will never reach human consciousness [10].

4.5. Animal Welfare and Dignity

Animal welfare is another bioethical dilemma raised by the use of genome editing technology on animals. Firstly, off-target mutations in the genome have the potential to cause illnesses or other negative consequences in animals. Such a situation will adversely affect animal welfare [7].

Another bioethical issue to be discussed could be the concerns about “animal dignity” and alterations in their natural environments and physiological needs. According to several studies, using animals as objects only for the benefit of humans is neither ethically nor morally acceptable, and such activities can lead to increased human control over animals. Some others say that animals are not bound by any moral law and hence there is no need for a discussion addressing animal dignity. Based on Schultz-Bergin (2017), the use of genome editing technology will have no negative impact on animal rights, welfare, or dignity. The existence of contrary opinions on this matter indicates that the mentioned bioethical issues will be on the agenda for a long time [7]. Crossing the species boundary between humans and animals is associated with additional ethical complexities that extend beyond traditional animal use. One of the main ethical concerns raised by human – animal chimeras is how to avoid stem cell contribution to the nervous or reproductive system. The possibility of inducing human cells into animal neural or reproductive systems has met particularly strong public resistance. For this reason, such research needs to be conducted cautiously, based on sufficient animal studies and under strict ethical review.

5. Conclusion

CRISPR technology is an innovative gene editing tool with high potential for improving plant and animal breeding systems in modern agriculture. This technology demonstrates significant advantages in areas such as increasing productivity, improving product quality, and strengthening resistance to biotic and abiotic stresses. In addition, the application of CRISPR raises a number of bioethical and biosafety issues, such as ecological balance, biodiversity conservation, consumer acceptance, animal welfare, and regulatory frameworks. It is necessary to expand scientific research and improve international regulatory mechanisms for safer and more effective application of this technology in the future. The application areas of this revolutionary technology are quite wide, and in the agricultural sector, it has already become possible to increase the tolerance of plant species to various pathogens, pests, as well as negative environmental stress factors such as drought and salinity through CRISPR.

Author Contributions

The author solely conceived and designed the study, conducted the literature review and analysis, interpreted the findings, drafted the manuscript, critically revised the content, and approved the final version of the manuscript for publication.

Conflict of Interest

The author declares no conflicts of interest.

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