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# Application of CRISPR-Cas technology in food safety

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**Abstract.** Food safety has become one of the most important public health issues in the world. Safety, nutrition, and food security are all interdependent. Unsafe food contributes to a cycle of illness and starvation that disproportionately affects young children, the elderly, and the ill. Therefore, it is essential to develop tests that are quick, efficient, and reliable. The CRISPR/Cas system is a bacterial acquired immune system that attacks invading DNA, plasmids, and phages. Genome editing using CRISPR/Cas offers new opportunities for plant breeding. Compared to animal cells, plant cells have rigid cell walls, making it challenging to deliver genome editing tools into plant cells. When using plants for industrial purposes, transgene insertion into the genome should be avoided. Therefore, delivery of Cas-gRNA ribonucleoprotein (RNP) into plant cells is preferred. This review proposed a novel RNP delivery strategy in rice and introduced a technology: whisker technology (commonly used for plant DNA delivery) to deliver RNPs into rice. This review also discussed ultrasound-assisted whisker technology via RNP management, combined with marker gene delivery, to identify genome editing events in rice decay cells in the absence of any other events, albeit at a lower frequency. Therefore, utilizing whiskers to generate RNP-based genome editing lineages in plants may be an attractive strategy.

**Keywords:** Food safety, CRISPR/Cas 9, gene-editing.

## 1. Introduction

Food safety is closely related to human health. Food allergy is a common problem in society in food safety. Start with the allergens and explore the causes of food allergies. For example, some people develop celiac disease due to wheat allergies (gluten stimulates the immune system to produce specific antibodies that damage the small intestinal mucosa and flatten the brush-likesurface layer of normal small intestinal villi). It is estimated that 600 million people globally (almost one in ten) become ill and die from contaminated food each year, resulting in the loss of 33 million healthy life years (DALYs). Because of contaminated food, low- and middle-income nations lose $110 billion in productivity and health expenditures each year. Children under the age of five face 40% of the burden of foodborne disease, with 125 000 deaths occurring each year. Foodborne infections put a load on health-care systems while also undermining national economies, tourism, and trade, stifling socioeconomic growth [1].

A research team from the Netherlands is currently trying to use CRISPR-CAS9 technology to modify the DNA of wheat so that it can be suitable for patients with celiac disease, and to explore the ingredients in different foods that cause human allergies. According to the investigation, it was learned that there are four proteins in egg whites that can cause allergies. (ovomucoid, ovalbumin, ovalbumin, lysozyme) [2]. The principle of PCR technology is to cycle back and forth through three steps of denaturation,

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renaturation, and extension of nucleic acid molecules. The target molecules are increased by multiples, so that trace amounts of measured molecules reach detectable levels. At present, PCR technology can be divided into three generations: traditional PCR (ePCR), fluorescent PCR and digital PCR, and on this basis, food safety detection technology has been extended. The current common PCR technology is to detect allergens and then prevent them. Compared with protein detection, its advantage is that it can still extract stable DNA under thermal denaturation. PCR technology is currently widely used in the detection food allergens. CRISPR-CAS9 technology is faster and more convenient than PCR technology. It can not only detect food-borne viruses efficiently, but also has great application prospects in food-borne viruses, genetically modified organisms, toxins, antibiotics and pesticide residues. If done correctly, the CSIRO is currently known to have begun using CRISPR-CAS9 technology to reprogramgenetic regions that cause allergies. Moreover, traditional breeding methods can take many years to develop new crop varieties with desired traits. CRISPR-Cas9 allows for precise and rapid gene editing, significantly speeding up the process of creating desirable crop varieties. The technology can also be used to enhance the quality and flavor of food products, leading to better-tasting and longer-lasting foods. Finally, by reducing the need for chemical pesticides and fertilizers, and by creating crops that are more resilient to environmental stressors, CRISPR-edited crops can contribute to more sustainable and environmentally friendly agricultural practices [3].

## 2. CRISPR/Cas9

Clustered Regularly Interspaced Short Palindromic Repeats and CRISPR-associated protein 9 are the abbreviations for CRISPR/Cas9, a groundbreaking genome-editing technology that enables exact DNA modification of an organism. It has gained immense attention and importance in the fields of genetics, molecular biology, and biotechnology [4].

During their long-term evolutionary process, bacteria and archaea developed the adaptive immune defense mechanism known as CRISPR-CAS9. This mechanism can be used to combat foreign DNA and viruses that invade the host. Bacteria incorporate plasmid DNA and phage fragments into CRISPR, and then transcribe and process them to generate the corresponding crRNA (CRISPR-derived RNA). The complex formed by crRNA combining with tracrRNA (trans-activating RNA) through base pairing can specifically recognize foreign substances.DNA double-strand breaks (DSBs) are created when the exogenous DNA is cleaved by the Cas9 endonuclease under the guidance of the source DNA sequence [5]. This causes the cells to repair themselves by homologous recombination or non-homologous end joining. In 2012, Dr. Jennifer Doudna, Dr. Emmanuelle Charpentier, and their colleagues made a pivotal breakthrough. They identified a specific Cas protein, called Cas9, as a versatile and programmable molecular scissors that could be guided to specific DNA sequences using a molecule known as RNA. This RNA-guided targeting system allowed precise gene editing.Since the initial discovery, scientists have continued to refine the CRISPR-Cas9 system and have developed various variants and improvements, such as Cas12 and Cas13, which allow for more precise and versatile gene editing (figure 1) [6].

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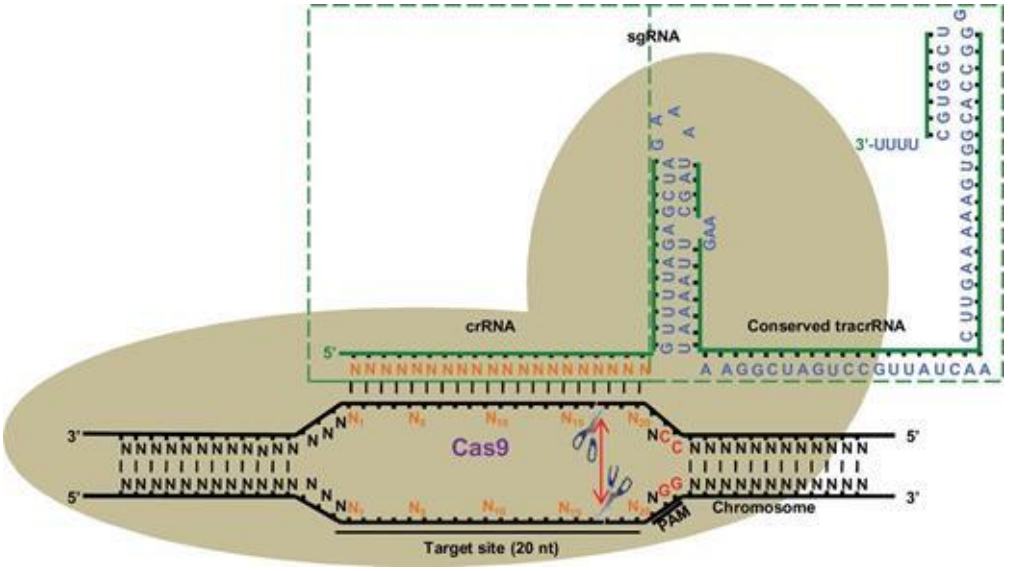


**Figure 1.** Recent advances of food safety detection by nucleic acid isothermal amplification integrated with CRISPR/Cas [7].

## 3. Application of CRISPR/Cas9 in food safety

In our daily life, we eat a lot of food. There is no shortage of genetically modified foods. Genetically modified potatoes and peanuts are plant species that people often grow, and they are also foods that people consume relatively large amounts of (figure 2). Arh 1 in peanuts is the main cause of allergies in patients with peanut allergens [7]. The principle of peanut allergy is that arh 1 binds to immunoglobulin E through the antigenic epitope, triggering allergy. The method of gene knockout in diploid cells was used in CRISPR-CAS9. Can inactivate the can 1 gene up to 4%. With a single guide RNA, the ribonucleoprotein of the Streptococcus pyogenes Cas9 protein selectively binds to DNA that contains the gRNA target sequence. At DNA target sites, ribonucleoproteins cause double-strand breaks [8]. The CRISPR technology's programmable features indicate significant promise for genomic breeding. Including promoters and terminators, the DNA fragments encoding CRISPR-Cas9 components and gRNA are frequently large, measuring more than 7 kbp. RNPs should be delivered directly to minimize the possibility of such large-sized DNA randomly integrating into the genome. RNPs are difficult to introduce into plant cells through the cell wall, in contrast to DNA delivery. Therefore, in recent years, the development of RNP delivery technology has attracted attention. In basic biological research, CRISPR-Cas9 is used to study the function of various genes, model diseases, and advance our understanding of genetics. In assisted reproductive technology, In order to prevent inherited genetic disorders, human embryos can have genetic mutations corrected or eliminated using CRISPR-Cas9 [9]. It's crucial to remember that CRISPR-Cas9 use is constrained by ethical and legal issues, especially when it comes to using it on people or producing genetically modified organisms (GMOs) [10]. To ensure that the benefits of technology are realized while minimizing potential risks and ethical concerns, responsible and transparent use of the technology is imperative [11].

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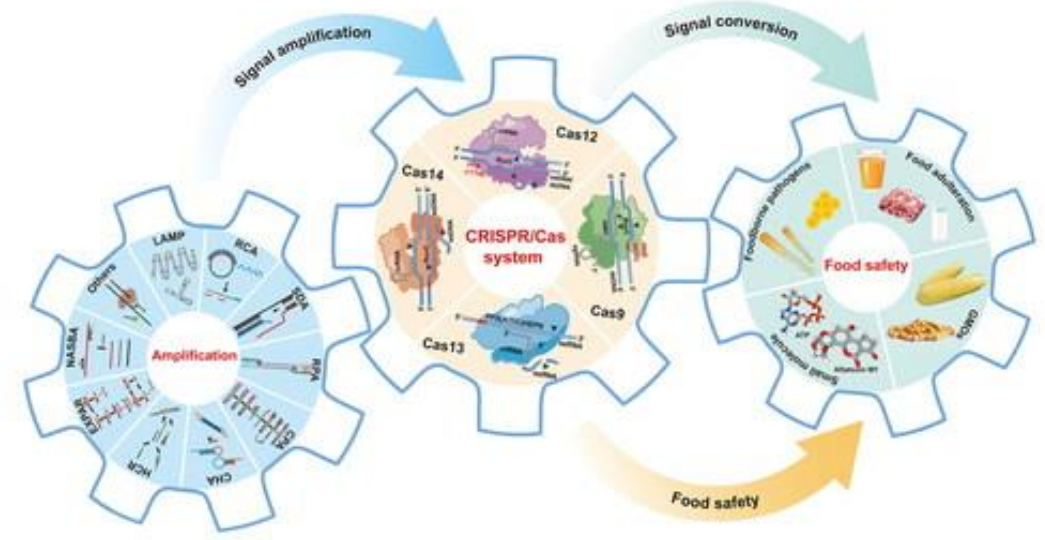


**Figure 2.** Recent advances of food safety detection by nucleic acid isothermal amplification integrated with CRISPR/Cas [1].

## 4. Conclusions

A novel gene-editing technique called CRISPR-Cas9 makes it possible to precisely alter an organism's DNA. An enzyme known as Cas9 is guided to particular DNA sequences by a molecule called RNA, which allows the enzyme to cut and modify genetic code. Thanks to this technology, genetics and molecular biology have undergone a revolutionary change that has made it possible to modify the genes of a wide variety of organisms, including humans. It can improve crop yields, treat genetic diseases, and do a lot more. However, it also raises ethical and regulatory concerns, particularly regarding the potential misuse of gene editing technology. As a result, responsible and careful use of CRISPR-Cas9 is a significant focus in its development and application. The application of CRISPR-CAS9 technology in food safety has received extensive support and research. According to the current technology, genome editing in rice is also expected to be realized. The currently known ultrasound-assisted beard technology will be very good. It has been applied in CRISPR-CAS9 technology, so CRISPR-CAS9 technology will have good application prospects in the future [12].

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