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Genetic Mechanism of Cassava Disease Resistance: From Traditional Breeding to CRISPR/Cas Application

Wenzhong Huang ✉, Zhongmei Hong
CRO Service Station, Sanya Tihitar Sci Tech Breeding Service Inc., Sanya, 572025, China
✉ Corresponding author email: hitar@hitar.org
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Abstract The escalating threat of plant diseases to cassava (*Manihot esculenta*) production necessitates innovative strategies for developing disease-resistant varieties. Traditional breeding has been instrumental in enhancing cassava's resistance to various pathogens, but it is often limited by the complexity of genetic traits and the lengthy timeframes required. The advent of CRISPR/Cas genome editing technology has revolutionized the field of plant breeding by enabling precise modifications of plant genomes. This systematic review provides a comprehensive analysis of the genetic mechanisms underlying cassava disease resistance and the transition from conventional breeding techniques to the cutting-edge CRISPR/Cas applications. We examine the current state of knowledge on plant-pathogen interactions in cassava and discuss how CRISPR/Cas-mediated genome editing has been employed to disrupt these interactions by targeting susceptibility factors within the plant genome. Furthermore, we explore the advancements in genome editing tools, such as base editing and prime editing, that have broadened the scope of generating disease-resistant cassava varieties. The review also highlights the potential of CRISPR/Cas9 in enhancing disease resistance through multiplexed gene editing and trait stacking, which is particularly relevant for complex traits like disease resistance. By synthesizing insights from recent developments in CRISPR/Cas applications across various crops, we aim to provide a roadmap for future research and the development of cassava varieties with improved resistance to a spectrum of diseases, thereby contributing to global food security.
Keywords Cassava disease resistance; Genetic breeding; CRISPR/Cas technology; Gene editing; disease management

Cassava (*Manihot esculenta*) is a staple crop that plays a pivotal role in the food security and economy of many tropical and subtropical regions. Its resilience to harsh environmental conditions and its versatile use in both human consumption and industrial applications underscore its agricultural value. However, the productivity and quality of cassava are severely threatened by various diseases, which can lead to significant economic losses and jeopardize food security. Understanding and improving cassava's disease resistance is therefore of paramount importance.

Diseases such as cassava mosaic disease (CMD) and cassava brown streak disease (CBSD) are among the most destructive, causing substantial yield reductions. Traditional breeding methods have been employed to enhance disease resistance in cassava, but these approaches are often time-consuming and limited by the availability of resistant germplasm. Moreover, the complex genetics of disease resistance traits can pose additional challenges to conventional breeding efforts (Zhu et al., 2020). The advent of modern gene editing technologies, particularly CRISPR/Cas systems, has revolutionized the field of plant breeding by enabling precise and targeted modifications to plant genomes (Shahriar et al., 2021).

CRISPR/Cas has emerged as a powerful tool for improving disease resistance in crops, including cassava, by editing susceptibility genes or by directly targeting pathogen genomes. This technology offers a rapid and efficient alternative to traditional breeding and has the potential to accelerate the development of disease-resistant cassava varieties.In this systematic review, we will explore the genetic mechanisms underlying cassava disease resistance, from traditional breeding practices to the cutting-edge CRISPR/Cas applications. We will delve into the current state of knowledge, recent advancements, and future prospects of employing gene editing for the enhancement of cassava's defense against its most threatening diseases.

1 The Main Diseases of Cassava and Traditional Disease Resistant Breeding

1.1 Main diseases of cassava

As an important food crop widely cultivated in tropical and subtropical regions, cassava is not only the staple food on the dining table of many people in many countries, but also deeply affects the economic structure and social well-being of these regions. However, the production of this crop faces many challenges, among which the most severe are diseases caused by various viruses, especially cassava mosaic disease (CMD) and African cassava mosaic virus (ACMDV), which belong to the dicotyledonoviridae family.

Cassava mosaic disease is a viral disease that can cause symptoms such as mosaic mottled leaves, reduced leaf size, and twisted deformation. In severe cases, it can also cause plant dwarfing, ultimately significantly reducing the yield and quality of root tubers. CMD is transmitted through mediating insects such as white flies, and is more easily spread in warm and humid environments, making cassava plantations in tropical regions particularly vulnerable. African cassava mosaic virus is a specific variant of CMD, with particularly significant destructive power on cassava crops. It can cause similar but often more severe symptoms, leading to widespread crop death and a more fatal impact on yield. The virus also relies on specific mediators for transmission, and in some areas, its impact is particularly prominent due to the lack of effective disease resistant varieties and management measures (Zhu et al., 2020).

1.2 Application and limitations of traditional breeding techniques in improving cassava disease resistance

Traditional breeding techniques have been employed to improve disease resistance in cassava. However, these methods face several challenges and limitations. Conventional breeding is often a lengthy process that may not keep pace with the rapid evolution of plant pathogens. Additionally, it relies on the availability of resistance genes within the gene pool of cassava, which may be limited or come with undesirable traits that are co-inherited during the breeding process. The precision of these traditional methods is also less than ideal, leading to a need for more advanced and targeted approaches to develop disease-resistant cassava varieties (Ahmad et al., 2020).

1.3 Breeding process and effectiveness of disease resistant varieties

The breeding process for disease-resistant cassava varieties involves the selection of parent plants that exhibit traits of resistance to specific diseases and then cross-breeding them to combine these traits in the progeny. The effectiveness of these disease-resistant varieties can be variable, as resistance may not be absolute and can be overcome by the pathogen over time. Moreover, the resistance bred into cassava must be carefully balanced with other agronomic traits to ensure that the resulting varieties are acceptable to farmers and consumers.

While traditional breeding has provided some disease-resistant cassava varieties, the limitations of this approach highlight the need for more innovative and precise genetic tools. The advent of CRISPR/Cas genome editing technology offers a promising alternative to overcome these challenges by enabling precise modifications to the plant genome, potentially leading to durable and robust disease resistance in cassava (Mehta et al., 2019).

2 Genetic Basis of Cassava Disease Resistance

2.1 Known cassava disease resistance related genes and genetic markers

Recent advancements in genetic research have identified specific regions and genetic markers associated with disease resistance in cassava. A genome-wide association mapping (GWAS) study has pinpointed two regions that are significantly associated with cassava brown streak disease (CBSD) resistance. One region on chromosome 4 coincides with a segment introgressed from Manihot glaziovii, while another on chromosome 11 contains a cluster of nucleotide-binding site-leucine-rich repeat (NBS-LRR) genes, which are known for their role in plant immune responses (Wheatley and Yang, 2020).

2.2 How disease resistance related genes affect the disease resistance of cassava

The NBS-LRR gene cluster identified on chromosome 11 is particularly noteworthy as these genes are a common type of resistance (R) gene in plants. They function by recognizing specific pathogen effectors and triggering defense mechanisms. The presence of these genes in cassava suggests a genetic basis for the plant's ability to recognize and respond to pathogens, thereby conferring resistance to diseases such as CBSD (Chavez-Granados et al., 2022).

2.3 The role and importance of genetic mechanisms in cassava disease resistance

The genetic mechanisms underlying disease resistance in cassava are crucial for the development of resistant varieties. Traditional breeding methods have been somewhat limited due to cassava's high heterozygosity and the separation of traits during breeding. However, genetic transformation and modern breeding techniques, such as CRISPR/Cas9, offer promising avenues for enhancing disease resistance in cassava. CRISPR/Cas9, in particular, allows for precise modifications of the cassava genome, potentially enabling the development of transgene-free, disease-resistant crops. This is a significant step forward in ensuring sustainable agricultural production and food security, as it accelerates resistance breeding and overcomes the limitations of conventional breeding (Li et al., 2021). The application of these genetic tools in breeding programs is expected to improve the resistance of cassava to various diseases, thereby contributing to the stability of food systems in regions where cassava is a critical crop (Mehta et al., 2019).

3 Introduction and Application of CRISPR/Cas Technology

3.1 Principles and operational steps of CRISPR/Cas technology

CRISPR/Cas technology is a revolutionary genome-editing tool that has transformed the field of genetics. The system originates from a natural defense mechanism found in bacteria and archaea, where it provides acquired resistance against viruses by targeting and cleaving their DNA (Tang et al., 2023). The CRISPR/Cas system is composed of two key components: the Cas9 enzyme, which acts as a molecular scissor, and a guide RNA (gRNA) that directs the Cas9 to a specific DNA sequence. The operational steps involve designing a gRNA that matches the target gene sequence, delivering the Cas9-gRNA complex into the organism's cells, and then relying on the cell's own repair mechanisms to introduce mutations or replace the gene sequence after the DNA is cut (Figure 1) (Tao et al., 2022).

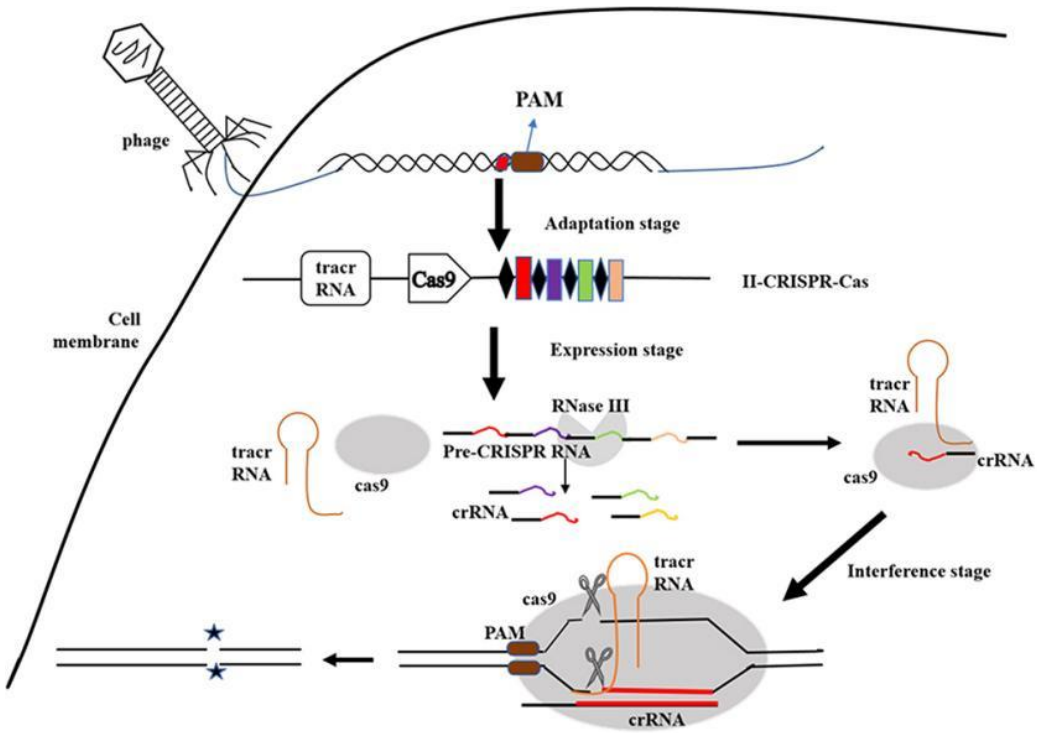

Figure 1 The working mechanism of the CRISPR/Cas system (Tao et al., 2022)

3.2 Application examples of CRISPR/Cas technology in cassava disease resistance

The application of CRISPR/Cas9 gene editing technology in cassava, an important tropical food crop, has opened up a new way for crop genetic improvement and enhanced disease resistance. By precisely modifying the genetic code of cassava, scientists are exploring how to use this revolutionary technology to combat various diseases that have long plagued cassava cultivation, such as those caused by viruses, fungi, and bacteria, which severely limit cassava yield and quality.

Researchers used the CRISPR/Cas9 system to target and edit the plant enolpyruvate dehydrogenase (MePDS) gene in cassava. The MePDS gene is involved in the synthesis pathway of chlorophyll. When the gene is knocked out or its function is impaired, plants will exhibit a clear albino phenotype, that is, the leaves lose their green color, which is due to the inhibition of chlorophyll synthesis. This easily recognizable phenotype has become an intuitive indicator for evaluating gene editing efficiency (Gomez et al., 2023). Through this approach, researchers have observed a high frequency of gene mutation events, including insertion, deletion, and point mutations of gene sequences. This not only demonstrates the high efficiency of CRISPR/Cas9 technology in cassava, but also provides the possibility for subsequent screening of mutant plants with disease resistance or other excellent traits.

More importantly, although MePDS editing itself does not directly confer disease resistance, it demonstrates how to precisely manipulate the genome of cassava through the CRISPR/Cas9 system. Based on this, scientists can further target genes directly associated with pathogen resistance, such as genes encoding disease resistant proteins or regulatory factors involved in plant immune responses. By precisely knocking out, modifying, or introducing new gene fragments, new varieties that can resist specific diseases can be created, such as those that can effectively combat cassava mosaic virus or African cassava mosaic virus (Tussipkan and Manabayeva, 2021).

The flexibility and accuracy of CRISPR/Cas9 technology also means that solutions can be customized for major diseases in different regions, thereby improving cassava's disease resistance and overall productivity on a global scale. With the continuous progress of gene editing technology and the expansion of its application scope, it is expected to see more gene edited cassava varieties in the future. Not only have strong disease resistance, but they may also have better nutritional value, stress tolerance, and higher yields, making significant contributions to ensuring food security in tropical regions and even globally.

3.3 Potential and challenges of improving cassava disease resistance through CRISPR/Cas technology

While CRISPR/Cas technology offers a promising avenue for enhancing disease resistance in cassava, there are several challenges and limitations to consider. The precision and robustness of CRISPR/Cas9 make it a valuable tool for crop improvement, but the development of transgene-free disease-resistant crops is still in its infancy (Cao et al., 2020). Resistance against CRISPR/Cas9 gene drive, a method to spread genetic modifications, is also a concern, as resistance can evolve almost inevitably in natural populations. This highlights the need for strategies to suppress resistance mechanisms or to use resistance as a control method. Despite these challenges, CRISPR/Cas technology holds great potential for the rapid and precise genetic improvement of cassava disease resistance, which is crucial for sustainable agricultural production and global food security (Cao et al., 2020).

4 Case Studies: Specific Application of CRISPR/Cas in Cassava Disease Resistance Breeding

4.1 A case study on using CRISPR/Cas technology to improve the disease resistance of cassava

The application of CRISPR/Cas technology in cassava disease resistance breeding has shown promising results. One specific case involves the targeting of susceptibility genes in cassava that are exploited by pathogens. By using CRISPR/Cas systems, researchers can knock out these genes, thereby disrupting the life cycle of the pathogen and conferring resistance to the plant (Schenke and Cai, 2020). Genes that facilitate the infection of cassava by viruses can be edited to create loss-of-function mutations, which in turn prevent the virus from successfully infecting the plant. This approach has been particularly effective against DNA viruses, where CRISPR/Cas9 can directly target viral sequences, and RNA viruses, where the technology is used to modify host plant genes to confer resistance (Robertson et al., 2022).

Another case study involves the use of CRISPR/Cas9 for the development of transgene-free disease-resistant crops. By targeting and editing endogenous genes within the cassava genome, researchers can enhance the plant's natural defense mechanisms without the introduction of foreign DNA, thus avoiding the regulatory hurdles associated with genetically modified organisms (GMOs) (Ahmad et al., 2020). This is particularly important for cassava, which is a staple food crop in many developing countries where GMOs may not be readily accepted.

4.2 Effectiveness and challenges faced by cases in practical applications

The effectiveness of CRISPR/Cas in improving cassava disease resistance is evident from the successful generation of plants with enhanced resistance to various pathogens. The precision and efficiency of CRISPR/Cas systems allow for the rapid development of disease-resistant varieties, which is crucial for a crop that is a key source of calories for millions of people. Moreover, the versatility of CRISPR/Cas tools enables the targeting of multiple genes or pathogens simultaneously, which is beneficial for breeding programs that aim to develop broad-spectrum disease resistance (Wheatley and Yang, 2020).

However, there are challenges in the practical application of CRISPR/Cas technology in cassava breeding. One major challenge is the delivery of CRISPR/Cas reagents into cassava cells, which can be difficult due to the plant's recalcitrant nature to transformation. Additionally, off-target effects, where CRISPR/Cas may inadvertently edit non-target regions of the genome, can pose a risk to the safety and stability of the edited plants. Regulatory challenges also exist, as the status of CRISPR-edited plants in terms of GMO legislation varies across different countries, potentially affecting the adoption and commercialization of CRISPR/Cas-derived cassava varieties (Tang et al., 2023).

5 Ethics, Law, and Social Acceptance

5.1 Ethical issues of gene editing technology in crop improvement.

The advent of CRISPR/Cas genome editing technology has brought about a significant shift in agricultural biotechnology and breeding, offering the potential to revolutionize modern agriculture. Despite its precision, ease of use, and cost-effectiveness, CRISPR/Cas technology raises ethical concerns due to the blurring of boundaries between natural organisms and genetically modified ones, leading to issues with the non-traceability of modifications. The ethical implications of gene editing in crops revolve around consumer and producer preferences, food ethics, and governance, necessitating a reevaluation of regulatory approaches to keep pace with technological advancements (Ntsomboh-Ntsefong et al., 2023).

5.2 Legal provisions and public acceptance of different countries regarding the use of CRISPR/Cas technology.

The legal landscape for CRISPR/Cas technology varies across countries, with some embracing the technology for its potential to improve crop yields and resilience to biotic and abiotic stresses, while others approach it with caution due to concerns about off-target effects and unintended consequences. Regulatory concerns are significant, as they address the safety and ethical considerations associated with genome editing. Public acceptance is also a critical factor, as it can influence the adoption and utilization of CRISPR/Cas technology in agriculture. The speed at which the technology is advancing poses a challenge to the regulatory processes, which often lag behind, leading to a gap between technological capabilities and societal debates (Ntsomboh-Ntsefong et al., 2023).

5.3 How these factors affect the application of CRISPR/Cas technology in cassava breeding

The application of CRISPR/Cas technology in cassava breeding is influenced by the ethical, legal, and social acceptance factors discussed above. Ethical concerns may lead to stricter regulations and oversight, potentially slowing down the adoption of CRISPR/Cas in crop improvement programs. Legal provisions can either facilitate or hinder the research and development of CRISPR/Cas-edited cassava varieties, depending on whether the regulations are enabling or restrictive. Public acceptance is crucial, as it can drive market demand for CRISPR/Cas-edited crops or lead to resistance against them, affecting the commercial viability of cassava varieties developed using this technology. Collectively, these factors play a pivotal role in determining the extent to which CRISPR/Cas technology can be applied to cassava breeding and the realization of its potential benefits in agriculture.

6 Outlook and Conclusion

The trajectory of cassava disease resistance research is poised for significant advancements, leveraging the synergy between traditional breeding methods and the revolutionary CRISPR/Cas technology. Traditional breeding has been instrumental in identifying and utilizing naturally occurring resistance, as evidenced by the discovery of a dominant gene conferring resistance to cassava mosaic disease (CMD), a major threat to cassava crops. This resistance, characterized as polygenic and later attributed to a major dominant gene CMD2, has been a cornerstone in breeding programs, particularly in Africa.

The advent of CRISPR/Cas9 genome editing has opened new horizons for enhancing disease resistance in cassava. This technology allows for precise modifications of the genome, enabling the development of transgene-free disease-resistant crops. CRISPR/Cas9 has been successfully applied to cassava, demonstrating its efficacy in targeted gene mutations to improve crop traits. Moreover, the technology has been used to edit the Phytoene desaturase (MePDS) gene in cassava, showcasing the high efficiency of CRISPR/Cas9 in inducing mutations and the potential for rapid assessment of genome editing.

However, the integration of CRISPR/Cas9 with traditional breeding faces challenges, including the evolution of resistance against CRISPR/Cas9 gene drive and the need for thorough investigation of its effects on plant physiological processes. Despite these challenges, CRISPR/Cas9 remains a transformative tool for plant breeding, with applications extending to the development of cultivars with hereditary resistance to diseases.

To better integrate traditional breeding techniques with CRISPR/Cas technology, researchers must focus on understanding the genetic basis of naturally occurring resistance and identifying susceptibility factors that can be targeted by CRISPR/Cas9. The use of CRISPR/Cas9 to introduce novel resistance genes or to edit existing ones offers a complementary strategy to traditional breeding, which relies on the selection of naturally resistant varieties.

Future research should prioritize the development of CRISPR/Cas9 systems that minimize the potential for resistance evolution, as well as the creation of computational tools for precise gene targeting. Additionally, efforts should be made to establish efficient plant regeneration protocols from protoplasts, particularly for woody species, to facilitate transgene-free editing.

The integration of traditional breeding and CRISPR/Cas technology represents a promising approach for developing disease-resistant cassava cultivars. Continued research is essential to overcome current limitations and to harness the full potential of CRISPR/Cas9 for sustainable agricultural production. The future of cassava disease resistance research is bright, with the potential to significantly improve crop resilience and contribute to global food security.

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Conflict of Interest Disclosure

The authors affirm that this research was conducted without any commercial or financial relationships that could be construed as a potential conflict of interest.

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