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Advances in Grapevine Disease Resistance: CRISPR/Cas9 Applications

Dandan Huang, Xingzhu Feng
Hainan Institute of Biotechnology, Haikou, 570206, Hainan, China
Corresponding email: xingzhu.feng@hibio.org
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Abstract This study collates the new progress in grape disease resistance, explains how CRISPR/Cas9 is used in the research and improvement of disease resistance genes, summarizes the pathogenesis of common grape diseases, and which disease resistance genes have been discovered and studied. It also analyzes some examples of improving grape disease resistance using CRISPR technology. This study also elaborates on the possible problems that CRISPR may encounter in the application of grapes, proposes some improvement methods for these problems, and discusses how gene editing can be combined with traditional breeding, how multiple traits can be improved simultaneously in the future, and how to better promote the market. This research aims to provide a scientific basis for the study of grape disease resistance and the breeding of more disease-resistant and market-popular grape varieties.
Keywords Grapes; Disease resistance; CRISPR/Cas9; Gene editing; Multi-omics integration; Breeding improvement

1 Introduction

The economic value of grapevine (*Vitis vinifera*) is very high. The grapevine industry holds a very important position in global agriculture. The production of wine, table grapes and raisins all contribute greatly to economic development. However, grapevines are vulnerable to various diseases, especially powdery mildew caused by the fungus *Erysiphe necator*, which is the most serious one. This disease can cause a significant decrease in yield. Many times, chemical fungicides are needed for control, but these agents may have adverse effects on the environment (Borrelli et al., 2018).

Disease-resistant breeding is a crucial step in making grapevine cultivation more stable and environmentally friendly. The traditional breeding methods are slow and not accurate enough. It is not easy to cultivate disease-resistant grapevine varieties. Erdoğan et al. (2023) found that genome editing technologies, especially the emergence of CRISPR/Cas9, have brought new solutions to this problem. This technology can directly and precisely modify the genes of grapevines, enhancing their disease resistance. Ren et al. (2022) believe that it is useful for reducing reliance on chemical agents and promoting more environmentally friendly agricultural methods.

The CRISPR/Cas9 technology is changing the way crops are bred by rapidly and precisely improving genes. This technology has been successfully used in grapevine research to edit susceptibility genes like *MLO*, making grapevines more resistant to powdery mildew. CRISPR/Cas9 is flexible and accurate, and has become a powerful tool for improving disease resistance and other important agronomic traits. Wan et al. (2020) also developed a method called “traceless editing”, which does not leave exogenous DNA and can also reduce regulatory issues related to genetically modified organisms, enabling this technology to be more widely applied.

This study will elaborate on the application of CRISPR/Cas9 technology in enhancing the disease resistance of grapevines, focusing on whether targeted mutations in susceptible genes can increase the resistance of grapevines to powdery mildew. This work is very important for grapevine cultivation. It can not only reduce the use of chemicals, but also possibly increase the yield and is more conducive to environmental protection. This research aims to provide assistance for the future cultivation of grapevine varieties that are more disease-resistant and more adaptable to the environment.

2 Current Status of Grape Disease Resistance Research

2.1 Major diseases of grapes and their pathogenic mechanisms

Grapevines are often threatened by fungal diseases such as *Plasmopara viticola* and *Erysiphe necator* during the cultivation process, and these diseases can affect the yield and quality of grapevines. Harper et al. (2023) demonstrated in their study that downy mildew often occurs in humid weather, causing leaves to be injured or fall off. Powdery mildew can infect the green parts of grapevines, leaving a layer of white powder on the surface, which looks like mold and affects the photosynthesis of the plants. Botrytis bunch rot caused by *Botrytis cinerea* can also cause economic losses. At present, it is mostly controlled by spraying fungicides, but this will make the germs drug-resistant. Fassolo et al. (2022) hold that to effectively prevent and control these diseases, it is necessary to understand how the pathogens invade grapevines and the defense mechanisms of the grapevines themselves.

2.2 Advances in research on disease resistance-related genes in grapes

Researchers have discovered many genetic loci related to the resistance of grapevines to downy mildew and powdery mildew in recent years. Liu et al. (2023) discovered that *Rpv36* and *Rpv37* are associated with downy mildew, and *Ren14* and *Ren15* are related to powdery mildew. These new loci are all concentrated in some genomic regions rich in disease-resistant related genes. Through transcriptome analysis, scientists have also identified different genes expressed when grapevines interact with pathogens, providing people with new insights into some secondary disease-resistant genes in grapevines.

2.3 Applications and limitations of conventional breeding techniques for disease resistance improvement

Traditional breeding methods often introduce disease-resistant genes from wild grapevines into cultivated varieties to enhance their disease resistance. However, this method has the problem of a long breeding cycle, and the expression of disease-resistant traits is greatly affected by environmental and pathogen changes, with a relatively complex phenotype (Ricciardi et al., 2024). Possamai and Wiedemann-Merdinoglu (2022) hold that some pathogens will gradually adapt to disease-resistant genes, which will affect the sustainability of the disease-resistant effect. At this point, long-term tracking and the adoption of multiple management methods to deal with it become necessary.

3 Application of CRISPR/Cas9 Technology in Grape Disease Resistance

3.1 Research on disease resistance gene mutation and regulation using CRISPR

The MLO gene family is the gene that makes grapevines prone to powdery mildew. Ahmad et al. (2020) edited these genes using CRISPR/Cas9 in their study. After adding some minor deletions or insertions to the two genes *VvMLO3* and *VvMLO4*, the resistance of some grapevine varieties to powdery mildew has become stronger. Paul et al. (2021) conducted a knockout experiment on the *VvPR4b* gene, which is related to the resistance of grapevines to downy mildew, using CRISPR/Cas9. They found that once this gene was knocked out, grapevines became more susceptible to downy mildew infection, indicating that *VvPR4b* is crucial in the disease resistance process (Figure 1).

3.2 Role of gene editing in functional studies of disease resistance-related genes

Gene editing with CRISPR/Cas9 is useful for a clearer understanding of the role of a certain gene in disease resistance. Researchers can observe whether the disease resistance of plants has changed by creating mutations with functional deficiencies and determine whether this gene is related to disease resistance. Alphonse et al. (2021) edited the *VvPR4b* gene and found that once this gene was knocked out, grapevines became more prone to downy mildew and the reactive oxygen species in their bodies also decreased, indicating that this gene is very important in disease prevention.

3.3 Case studies: improving grape disease resistance through gene editing

In 2022, Jiao et al. ’s research significantly enhanced the resistance of grapevines to powdery mildew through targeted mutations in the *VvMLO3* and *VvMLO4* genes. These mutated grapevines exhibit more cell death and cell wall deposition, which is a response of plant defense. Another study used the CRISPR immune system to combat grapevine leafroll-associated virus 3 (GLRaV-3). Li et al. (2020) successfully cultivated transgenic grapevine varieties with strong antiviral capabilities by using CRISPR/FnCas9 and LshCas13a.

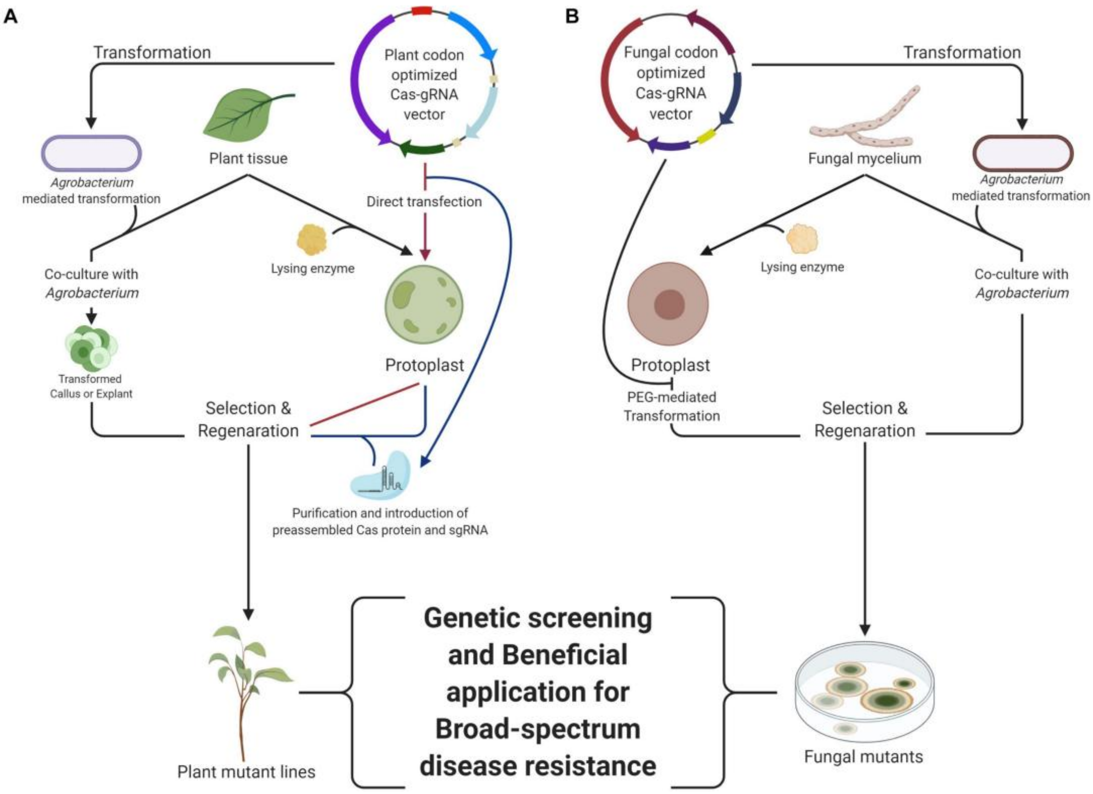

Figure 1 Workflow of CRISPR/Cas system in plants (A) and fungi (B); Agrobacterium-mediated transformation is a common method for genome modification in plants, including CRISPR/Cas system delivery (Adopted from Paul et al., 2021)

4 Technical Optimization and Challenges

4.1 Technical bottlenecks in applying CRISPR/cas9 technology to grapes

Najafi et al. (2022) demonstrated that the fact that grapevines are woody plants makes gene editing more complex, especially in multicellular explants such as somatic cell embryos, where there is still a lack of efficient gene transfer methods, which is one of the main obstacles. Unstable editing efficiency is also a big problem. Ren et al. (2024) found that the editing efficiency of the same CRISPR/Cas9 system might vary from 0% to 38.5% in different experiments. Such a significant difference indicates that the transmission mode and expression system of CRISPR still need to be further improved to enable it to exert a more stable effect in grapevines.

4.2 Off-target effects and genetic stability issues in gene editing

Off-target effects are often encountered in the application of CRISPR/Cas9 technology. Sometimes the system may act in places where it shouldn't be edited, which may cause genes to become unstable. Such mistakes will affect the development of disease-resistant grapevines, as inaccurate editing may cause side effects. Osakabe et al. (2018) attempted to solve this problem by using shorter sgRNA or dual Cas9 nickases, and these methods have been proven to reduce unexpected modifications. Researchers also pay attention to whether the edited plants are stable, because some edited grapevines may have problems such as yellowing and necrosis of leaves, which affect the breeding effect.

4.3 Methods to improve the efficiency of gene editing

Researchers have tried many methods to achieve better gene editing effects of CRISPR/Cas9 in grapevines. Olivares et al. 's research in 2021 demonstrated that the use of geminivirus-based replicons can enhance the expression efficiency of CRISPR/Cas9 and achieve better editing results in multicellular explants. The method of directly delivering CRISPR/Cas9 ribonucleoproteins (RNPs) into protoplasts is considered to have great development prospects. It does not require the integration of exogenous DNA and can avoid regulatory issues related to genetically modified organisms. Nyu (2024) holds that adjusting the ratio of Cas9 to sgRNA is also crucial. An appropriate ratio can enhance the transformation efficiency of protoplasts and the success rate of targeted mutations (Figure 2) (Olivares et al., 2021).

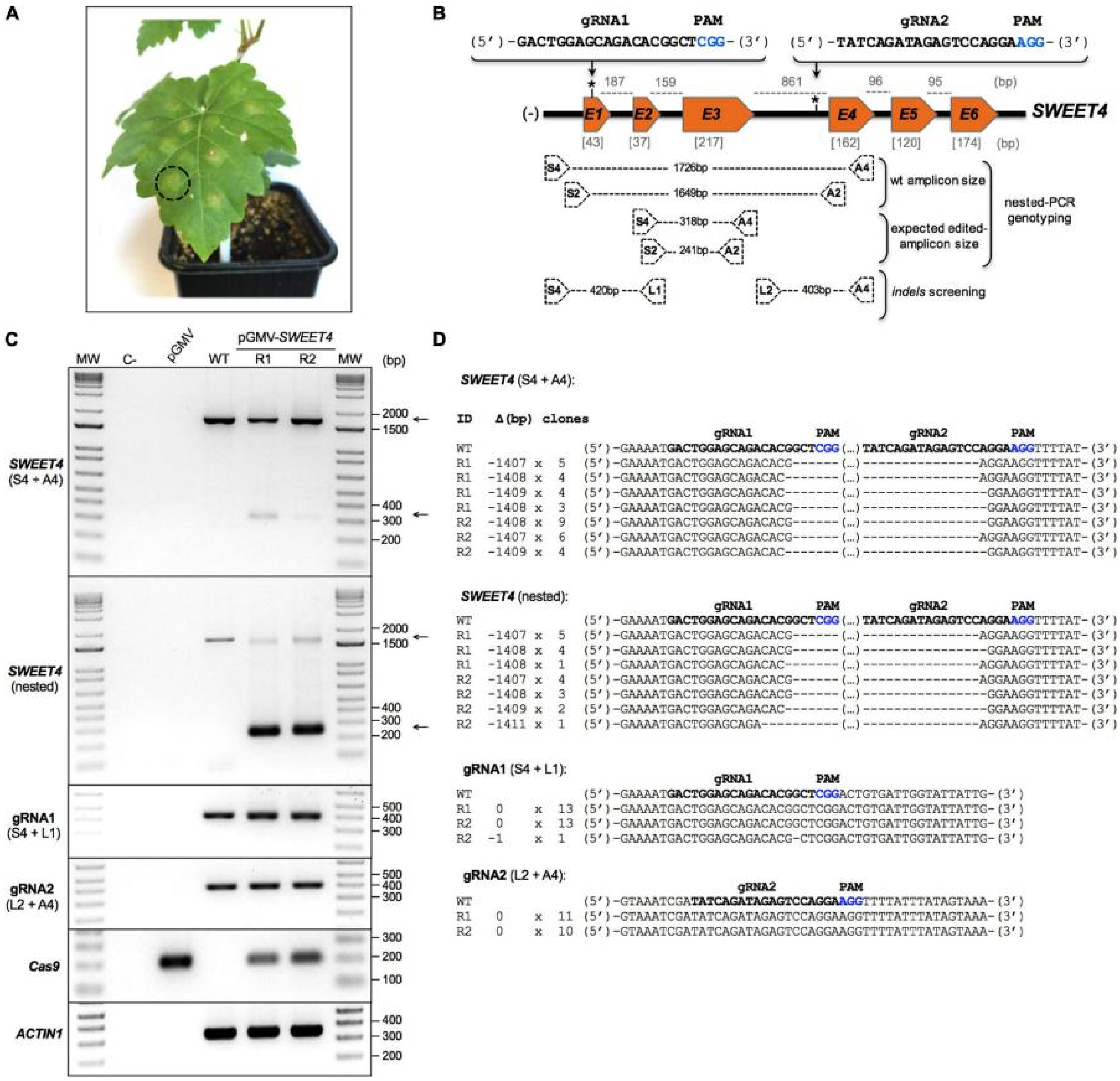

Figure 2 CRISPR/Cas 9-Mediated gene editing in grapes using Agrobacterium (Adopted from Olivares et al., 2021)

5 Discovery and Application Prospects of Disease Resistance Genes in Grapes

5.1 Multi-omics studies and integrated analysis of disease resistance genes

Karn et al. (2021) discovered some genes with significant expression changes in *Vitis vinifera* through transcriptome sequencing, and identified pathways related to disease resistance such as phenylpropane biosynthesis and the MAPK signaling pathway. Another study has discovered a disease-resistant gene locus called *REN12* in *Vitis amurensis*. It can prevent the spread of the disease when the pathogen just begins to infect and shows strong resistance. These studies indicate that combining different types of data is useful for discovering more genes involved in the disease resistance process, especially those secondary resistance genes (Su et al., 2023).

5.2 Phenotypic validation and molecular mechanism analysis of edited disease resistance genes

Confirming whether disease-resistant genes are truly effective usually requires phenotypic verification, that is, testing the disease-resistant performance of these genes under different conditions. Sapkota et al. (2023) recently found in their research that plants' resistance to powdery mildew increased after overexpressing the *VqSERK3/BAK1* gene in *Arabidopsis thaliana*, as this gene can regulate cell death and stomatal immune responses.

The *REN11* disease resistance site from *Vitis aestivalis* has shown stable powdery mildew resistance under various environmental conditions. Conducting phenotypic verification not only makes the mechanism of action of these genes clearer, but also enables them to be better applied to grapevine breeding.

5.3 Functional stability studies of disease resistance genes across different grape cultivars

The key to whether disease-resistant genes can play a stable role in different grapevine varieties for a long time lies in whether they can be truly applied in breeding. Yin et al. (2022) found that resistance sites like *REN11* remain effective in various tissues and environments, indicating that it holds promise for long-term and stable disease-resistant breeding. Yan et al. (2017) indicated that some disease-resistant genes are only effective in specific tissues or specific environments, and certain QTLS related to fungal diseases exhibit such characteristics in grapevines. For disease-resistant genes to be truly widely applied, more comprehensive studies on their stability and adaptability are needed.

6 Future Directions for CRISPR/Cas9 in Grape Breeding

6.1 Integration of gene editing with conventional breeding strategies

The combination of CRISPR/Cas9 gene editing technology and traditional breeding methods brings great hope for the improvement of grapevine varieties (Zhong, 2024). This method can cultivate new varieties with strong disease resistance, good fruit quality and high yield more quickly. It can precisely modify specific genes and retain the genetic diversity and stress resistance brought about in traditional breeding (Wang et al., 2017). Ren et al. (2019b) argued that CRISPR/Cas9 can also specifically control those genes that cause adverse traits to make up for the insufficiently precise selection process in traditional breeding.

6.2 Development of gene editing techniques for multi-trait improvement

The CRISPR/Cas9 technology can now edit multiple genes simultaneously, which is crucial for improving the complex traits controlled by multiple genes in grapevines. Fizikova et al. 's research in 2021 found that multiple editing techniques can jointly enhance traits such as disease resistance, fruit quality, and stress tolerance. In grapevine research, there have been successful examples of developing efficient multi-editing systems, which makes it possible to simultaneously improve multiple target traits and cultivate excellent grapevine varieties with multiple advantages at the same time.

6.3 Public policy and market promotion potential for gene-edited grapes

Whether gene-edited grapevines can be widely accepted and commercialized largely depends on policy support and market promotion methods. The resolution of related regulatory and public awareness issues has become increasingly important with the development of CRISPR/Cas9 technology. Zhou et al. (2020) demonstrated that “traceless” editing technologies like CRISPR/Cas9 ribonucleoprotein do not introduce exogenous DNA and are expected to bypass some regulatory obstacles related to genetically modified organisms. Good communication methods are also needed. Only by making the public aware of the benefits and safety of gene-edited grapevines can we win their trust and make it easier for these new varieties to enter the market.

7 Data Integration and Collaborative Mechanisms

7.1 Sharing and integration of multi-omics data in disease resistance research

Chao et al. (2023) proposed in their study that methods such as genomics, transcriptomics, and metabolomics are beneficial for a more comprehensive understanding of plant traits and their interactions. A large amount of data has been accumulated at present, but due to different sources and the lack of unified standards for metadata, it is still very difficult to fully integrate these data. Conesa and Beck (2019) discovered that the TransMetaDb database in the Vitis Visualization platform is promoting data sharing and integration. These platforms enable researchers to conduct more in-depth analyses such as studies on the relationships between genes and between genes and metabolites, providing strong support for scientific research and grapevine breeding.

7.2 Importance of international collaboration in grape disease resistance research

Grape cultivation is important in many parts of the world, and similar problems are also encountered in disease resistance research. Through international cooperation, researchers can share resources, technologies and data together to accelerate the process of cultivating disease-resistant varieties. Brilli et al. (2018) demonstrated that the COST Action CA17111 INTEGRAPE project is a good example, which shows that international cooperation is helpful for establishing shared resources and databases that are convenient for the entire research community to use. International cooperation can also promote the unification of research methods and data formats, which is crucial for subsequent data integration and utilization.

7.3 Role of open-access databases in promoting gene-edited grape breeding

Open-access databases provide researchers with a lot of useful genetic and phenotypic data. Savoi et al. (2022) hold that these databases enable the sharing of multi-omics data, which is beneficial for identifying target genes and verifying their functions, thereby accelerating the application of gene editing in breeding. By integrating different types of data, researchers can have a clearer understanding of how molecules interact with each other in disease resistance, making breeding programs more efficient. When these databases are findable, accessible, interoperable and reusable (F.A.I.R.), they can provide great assistance for the innovation of breeding strategies and the rapid promotion of research results.

8 Concluding Remarks

The CRISPR/Cas9 technology has promoted the development of grape disease resistance research by precisely modifying genes. The targeted mutation of the *VvMLO3* gene has enhanced the resistance of grapevines to powdery mildew, which would otherwise seriously affect the yield. This technology can modify the genes that make plants prone to diseases, reduce the chance of infection, and is also helpful in improving the overall health level of grape plants. CRISPR is also used to enhance the resistance of grapevine leafroll-associated virus 3 (GLRaV-3), indicating that it can deal with various grapevine diseases and has a wide range of applications.

CRISPR technology can be used in grapevine breeding to improve disease resistance, increase yield, enhance fruit quality, and strengthen the adaptability to climate change, etc. It can produce non-genetically modified mutants without introducing foreign DNA and simultaneously edit multiple alleles in polyploid genomes, making it a powerful tool for precision breeding. With the continuous advancement of technology, it is expected that in the future, grapevine varieties that can resist current diseases and also meet the new challenges brought about by climate change will be cultivated. New CRISPR tools such as Prime Editing are also expected to improve the accuracy and efficiency of editing.

This study highlights the significant role of CRISPR/Cas9 technology in enhancing the disease resistance of grapevines and demonstrates its great potential in promoting green agriculture. By reducing the use of pesticides and enhancing the immunity of plants themselves, CRISPR helps achieve more environmentally friendly planting methods and is also conducive to ensuring food security. Its successful application on grapevines provides a reference for other fruit tree crops, making the promotion of gene editing technology in agriculture easier. In the future, as countries gradually follow suit in terms of regulations, CRISPR technology is likely to become a regular tool in breeding programs, promoting the more efficient and resilient development of agriculture.

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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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