

Research Insights

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Genomic Strategies for Disease Resistance Breeding in Sugarcane: Identification of Resistance Genes, Transcriptomic Analysis, and Molecular Markers

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Preferred citation for this article:

Huang D.D., and Wang H.M., 2025, Genomic Strategies for disease resistance breeding in sugarcane: identification of resistance genes, transcriptomic analysis, and molecular markerst, Bioscience Methods, 16(3): 108-116 (doi: 10.5376/bm.2025.16.0011)

Abstract Sugar cane is an important sugar crop in the world. Its yield and quality are often affected by a variety of diseases, which seriously restricts industrial development. Traditional disease-resistant breeding methods have problems such as long cycles and low efficiency, which are difficult to meet the needs of modern agriculture for efficient and precise breeding. With the advancement of genomics technology, sugarcane disease-resistant breeding has entered a new stage. This study systematically reviews the genomic strategies of sugarcane disease-resistant breeding, covering genomic resource construction, disease-resistant gene mining and functional verification, transcriptome analysis, molecular marker development, and multiomic integration application. Particularly emphasized the potential of emerging technologies such as gene editing, pan-genome and artificial intelligence in improving the efficiency of disease-resistant breeding. By integrating multi-level omics data and advanced technologies, sugarcane disease-resistant breeding is expected to achieve the transformation from traditional experience to precise design, providing a solid theoretical foundation and technical support for cultivating new sugarcane varieties with high yield, high sugar and disease-resistant sugarcane. **Keywords** Sugarcane; Disease-resistant breeding; Genomics; Transcriptome analysis; Molecular marker

1 Introduction

Sugarcane (*Saccharum* spp.) is an important sugar and bioenergy crop worldwide and is mainly grown in tropical and subtropical regions. However, various diseases seriously threaten their production, causing significant economic losses. To achieve sustainable cultivation and meet the growing demand for sugar and biofuels, it is especially important to cultivate disease-resistant sugar cane varieties.

Sugarcane normally gets sick because of different germs like fungi, bacteria and viruses. The most common diseases are red rot disease, smut disease, white leaf disease and mosaic disease. Sugarcane plants do not mature well if they are infected by these diseases. As a result, farmers harvest a lot less sugarcane and its quality is also poor. This has proved to be a major problem for sugarcane fields across the globe, as it is now harder to grow enough healthy sugarcane (Pimenta et al., 2023).

Because sugarcane has a complex polyploid genome, traditional disease-resistant breeding methods face many difficulties. This complexity increases the difficulty of genetic analysis and extends the breeding cycle (Wu et al., 2022). Furthermore, disease-resistant traits have quantitative genetic characteristics and are susceptible to environmental factors, making it difficult to obtain stable and lasting disease resistance by conventional methods (Lu et al., 2023; Lin et al., 2024).

New gene technology is helping make sugarcane more disease-resistant. Scientists can now use DNA sequencing to find genes that fight diseases. This helps pick the best plants for breeding faster. Another method called GWAS looks at the whole genome to spot disease-resistant traits. By studying how genes work when plants get sick, we learn how sugarcane defends itself. Adding these gene tools to breeding programs speeds up creating stronger sugarcane types. This is good for farming long-term because healthier plants mean better harvests.



2 Sugarcane Genomic Resources and Identification of Disease Resistance Genes

2.1 Complexity of the sugarcane genome and recent assembly achievements

Sugarcane harbors highly complex DNA that is difficult to handle. Scientists have identified some vital sets of genes that make the plant disease resistant. Experiments show that NBS-LRR genes are the key to ensuring sugarcane's immunity to infections. Wild sugarcane species, especially *Saccharum spontaneum*, contribute precious disease-resistance genes to the cultivated sugarcane. Researchers have also cloned catalase genes that make it possible for sugarcane to cope with stresses in the environment. These results reveal how sugarcane's complex genetic makeup enables it to survive. The findings indicate the value of wild sugarcane types for breeding hardier, disease-resistant crops (Jiang et al., 2023).

2.2 Identification and annotation of disease-related gene families

Researchers have identified a number of disease-resistant genes in sugarcane using various genetic analysis methods (Parvaiz et al., 2021). One of the significant discoveries is resistance gene analogues (RGAs) - these genes have differential patterns of activity in disease-resistant sugarcane compared to susceptible ones, clearly demonstrating their protective role (Rody et al., 2019). Recent studies with improved genetic network analysis identified interrelated groups of genes involved in smut disease prevention as a group and in plant stress alleviation (Wu et al., 2022). These all give the insight into sugarcane's natural defense mechanisms. The research shows how certain genetic studies result in the discovery of sugarcane's innate protection networks, providing prospective knowledge for creating more resistant varieties of sugarcane.

2.3 Functional validation and expression analysis of resistance genes

Functional verification and gene expression analysis are very critical to understanding the mechanism of action of disease-resistant genes. Studies have found that some genes (such as *ScCAT1*) are upregulated under pathogen stress, which can enhance disease resistance by regulating reactive oxygen levels (Wu et al., 2023). In addition, the expression of some specific disease-resistant genes (such as *PR10* and *HCT1*) has been shown to be related to early infection processes and disease-resistant mechanisms (Hidayah et al., 2021). These findings emphasize the importance of functional verification in identifying key disease-resistant genes and also provide the possibility of its application in breeding.

3 Application of Transcriptome Analysis in Studying Disease Resistance Mechanisms 3.1 Transcriptomic changes in sugarcane under pathogen infection

Transcriptome analysis plays an important role in revealing the response mechanism of sugarcane to pathogenic infection. For example, during *Sporisorium scitamineum* infection, studies have found a large number of differentially expressed genes (DEGs), showing a complex stress response mechanism in sugarcane (McNeil et al., 2018). Similarly, many DEGs associated with metabolic processes and phytohormone signaling were also detected after *Xanthomonas albilineans* infection, which are crucial for plant defense responses (Figure 1). These studies show that sugarcane has dynamic changes in its gene expression during its resistance to pathogen invasion.

3.2 Screening and functional classification of differentially expressed genes

Scientists have been studying how sugarcane genes respond when the plant is sick. They have noticed that there are certain genes that get expressed when disease hits. These genes are associated with certain important defense responses in the plant. There are certain genes that act through stress response pathways. Some of these include the MAPK signaling pathway that functions as an alarm system, hormone signaling pathways, and pathways that assist the plant in fighting germs (Ntambo et al., 2019). Scientists found out that most of these active genes assist in making special chemicals that combat diseases. These are crucial for protecting the plant. In a surprising turn of events, even genes that regulate basic plant processes seem to play a role in defense. These are photosynthesis genes (the process by which plants generate food from sun energy) and amino acid biosynthesis genes (protein precursors) (Zhang et al., 2022). These findings confirm that sugarcane utilizes many different systems to protect itself against disease, along with its special defense genes. The plant's normal processes also help keep it in good health.





Figure 1 Transcriptome analysis of genes in the RNA-seq dataset of sugarcane (Adopted from Jiang et al., 2023) Image caption: Transcriptomic analysis in multiple RNA-seq datasets. (A) Differential expression analysis of *S. spontaneum* and *S. officinarum* alleles. (B) Differentially expressed NBS-LRR genes in multiple diseases. (Adopted from Jiang et al., 2023)

3.3 Construction and analysis of key disease resistance pathways

Researchers are studying how the synergy of sugarcane genes operates when they resist disease. Through one method called WGCNA, sets of genes were discovered to collaborate in resisting smut disease (Wu et al., 2022). These sets of genes control critical processes in the plant like the synthesis of glutathione and flavonoids that function as a protective role. By comparing gene activity data to biological networks, scientists have gained more insight about disease-resistance genes called RGAs and how they work with other defense systems (Rody et al., 2021). The study is revealing sugarcane's natural defense while helping to decide on the best genes to employ in breeding healthier, disease-resistant sugarcane. The study provides vital information for breeding sugarcane that will be more resistant to disease but also maintain quality production.

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4 Development of Molecular Markers and Their Application in Disease-Resistant Breeding 4.1 Development of SNP and SSR markers associated with disease resistance

The development of molecular markers such as single nucleotide polymorphisms (SNPs) and simple sequence repeats (SSRs) has greatly promoted the development of disease-resistant breeding. In cassava, researchers have developed and verified some SNP markers associated with cassava mosaic disease resistance sites that have high prediction accuracy against traits (Ige et al., 2021). Similarly, SSR markers associated with resistance to multiple diseases were also found in other crops. For example, SSR markers in peanuts are associated with resistance to premature spot disease, SSR markers in rice are associated with resistance to brown planthoppers (Shabanimofrad et al., 2015), while SSR markers in cotton are associated with resistance to jassid (Venkatesulu et al., 2023). These markers are of great significance for identifying disease-resistant genotypes and promoting the application of marker-assisted selection (MAS) in breeding.

4.2 Association analysis between markers and disease resistance traits

Researchers use correlation analysis to link DNA markers to disease resistance in plants. They discovered specific SSR markers linked to early spot disease resistance in peanuts, which helps breeders develop better varieties (Zongo et al., 2017). Research on rice showed the same SSR markers linked to resistance to brown planthoppers, explaining some of the defensive traits of the plants. Tobacco researchers also discovered SSR markers linked to resistance to potato Y virus, enabling breeders to select resistant plants using novel tools (Darvishzadeh et al., 2016). The marker research helps to confirm which genetic markers truly predict disease resistance and whether they work in real breeding programs so that crop improvement can become more precise and efficient.

4.3 Application of marker-assisted selection in disease-resistant breeding

Marker-assisted selection (MAS) has become a core tool in disease-resistant breeding programs. In rice, SSR-labeled MAS was successfully selected to breed species that resist brown planthoppers (Shabanimofrad et al., 2015). In cassava, SNP markers are used to accelerate the introduction of resistance alleles such as mosaicism in breeding populations (Ige et al., 2021). In addition, SSR markers are also used for selective breeding in shrimp breeding to enhance their resistance to viral and bacterial pathogens (Yin et al., 2023). These applications show that MAS has significant results in improving the disease resistance of crops and breeding species and accelerating the breeding process.

5 Integration of Multi-Omics and Optimization of Disease-Resistant Breeding Strategies 5.1 Integrated analysis of genomic and transcriptomic data

Scientists are linking genetic and gene expression data to better comprehend how sugarcane can resist disease. Scientists could find useful disease-resistance genes and how they work by studying the DNA of the plant as well as what genes are turned on when infected (Pimenta et al., 2023). For example, to evaluate mosaic virus resistance, they applied GWAS and RNA sequencing for gene network assembly, which showed that genes of photosynthesis and stress response are involved in the plant defense. Another smut disease research utilized WGCWA to uncover how groups of genes interact with each other during disease infection, stress-related genes being an important feature (Wu et al., 2022). These complementing methods offer a better picture of the natural resistance of sugarcane and allow scientists to ascertain the most valuable genes to utilize in breeding healthier, disease-free sugarcane varieties.



5.2 Application of proteomics and metabolomics in studying disease resistance mechanisms

Proteomics and metabolomics provide a new perspective for studying the pathogenesis of sugarcane. Proteome studies have identified a variety of differentially expressed proteins (DEPs) that play an important role in disease resistance, including defense-related proteins that deal with pathogens such as *Sporisorium scitamineum* and *Acidovorax avenae* (Su et al., 2016). For example, there are significant expression differences in proteins related to metabolic processes, stress responses, and defense in disease-resistant and sensory sugarcane varieties, which may become potential disease-resistant biomarkers (Singh et al., 2019; Zhou et al., 2021). Although metabolomics is less studied, it has a complementary role in revealing the metabolic pathways activated during pathogen infestation, helping to further understand the biochemical basis of disease resistance.

5.3 Multi-omics data-driven strategies for disease-resistant breeding

Integrating multiomic data such as genome, transcriptome, proteome and metabolomic is the key to formulating efficient disease-resistant breeding strategies. Through multidimensional data integration, researchers can identify a series of core disease-resistant genes, regulatory pathways and biomarkers, thereby providing strong support for breeding programs (Chen et al., 2024). For example, the integration of genomic and transcriptomic data plays an important role in identifying molecular markers associated with resistance to puff blight (Pokkah Boeng) and sugarcane yellow leaf virus (SCYLV), providing a key tool for marker-assisted selection (MAS) (Pimenta et al., 2021; Lin et al., 2024). In addition, a genomic prediction model combining machine learning and feature selection improves the accuracy of prediction of disease-resistant traits, providing a practical solution for the breeding of disease-resistant sugarcane varieties (Islam et al., 2021; Pimenta et al., 2023). These multiomic strategies not only help to have a deep understanding of the pathogenesis of sugarcane, but also accelerate the cultivation process of strongly resistant sugarcane varieties (Li, 2024b).

6 Prospects of Emerging Technologies in Sugarcane Disease-Resistant Breeding

6.1 Potential applications of gene editing technologies

Gene editing technology, especially CRISPR/Cas9, is innovating the field of plant disease-resistant breeding. Compared with traditional gene editing tools such as megnucleases, zinc finger nucleases (ZFNs) and transcriptional activator-like effector nucleases (TALENs), CRISPR/Cas9 has the advantages of simple design, high success rate, wide application range and low cost, so it is more popular (Borrelli et al., 2018; Boubakri, 2023). This technology can accurately modify the plant genome, and cultivate disease-resistant crops by targeting and regulating susceptible genes. For example, CRISPR/Cas9 has been successfully applied to crops such as rice, tomato and wheat to enhance their resistance to virus, fungal and bacterial diseases (Park et al., 2024). In sugarcane breeding, the potential of CRISPR/Cas9 lies in its ability to create disease-resistant varieties that do not require genetically modified, which is of great significance for achieving sustainable agricultural development (Ahmad et al., 2020). However, gene editing technology also has many limitations (Figure 2).

6.2 Prospects of pan-genomics and single-cell omics in disease resistance research

Researchers are applying new approaches called pangenomics and single-cell omics to investigate how plants defend against disease. The approaches enable the comparison of genetic variation among plant species and the identification of genes that enhance crop disease resistance. Researchers also integrate various approaches-screening genes, proteins, and metabolites in plants-to understand how crops react to environmental stressors (Razzaq et al., 2021). Utilization of these techniques in combination with the CRISPR gene-editing tool enables the study of particular genes and enhances crop resistance to pathogens. This strategy has the potential to develop novel and improved ways of safeguarding sugarcane from infection. The new technologies give researchers an insight into plant defense that was not possible with the previous techniques.

6.3 Application of artificial intelligence and big data in predicting disease resistance genes

Artificial intelligence and big data are transforming learning on plant breeding, particularly the identification of disease-resistant genes. Such emerging technologies can analyze vast amounts of plant trait and genetic data and identify patterns that human methods previously overlooked. Smart computer algorithms search difficult data to make informed guesses about how genes interact with one another, thereby making it simpler for scientists to



identify new disease-fighting genes more quickly. When researchers combine AI with genetic data, breeding accelerates and yields more insights into how crops display disease resistance (Li, 2024a). The process has been especially helpful in breeding disease-resistant sugarcane.

Limitations of CRISPR/Cas9
Bigger Protein size
Limited PAM sites
More off-target mutations
Low HDR efficiency
Low-efficiency against viruses
Costly and time consuming due to Agrobacterium-mediated transformation system
Hindrance in commercialization of newly developed CRISPR-edited crops due to ethical issues and rules and regulations of different countries

Figure 2 Limitations of the CRISPR/Cas9 system (Adopted from Ahmad et al., 2020)

7 Concluding Remarks

Genome strategies have become an indispensable and important means in breeding of disease-resistant sugarcane varieties. The integration of genome selection and genome-wide association analysis (GWAS) greatly promotes the identification of disease-resistant genes and molecular markers, providing key support for the cultivation of disease-resistant varieties. For example, genomic selection has been shown to improve the accuracy of predictions for resistance traits such as brown rust and orange rust, where nonparametric models outperform parametric models, indicating an important role of non-additive genetic effects in disease resistance. In addition, the identification of single nucleotide polymorphism (SNP) sites associated with resistance such as sugarcane mosaic virus (SCMV) and red rot also provides practical pathways for marker assisted breeding. These progress highlights the important role of genomics in accelerating the breeding process and enhancing sugarcane disease resistance.

Researchers are using a mix of various techniques to improve sugarcane breeding. By combining genetic studies, gene expression, and computerized machine learning, they're able to identify better how sugarcane defends itself against disease. For instance, research that examined which genes are expressed when the plant is under disease infection found important genes for photosynthesis and stress response that fight sugarcane mosaic virus. Computer programs are also helping to pinpoint precisely where in the sugarcane DNA the disease resistance is encoded so that it can be easily recognized and predicted such factors as brown rust resistance. If scientists collect all this diverse data, they can choose the most appropriate plants to breed much faster, leading to sugarcane varieties that stay healthier.



Scientists who study disease-resistant sugarcane have to concentrate on several important things. First, they need to study more diseases. At the moment, scientists only study three important diseases: SCMV, red rot, and brown rust. By studying more diseases, we can learn more about how sugarcane fights infection. Scientists need to create improved computer programs to sort out which plants of sugarcane will be most resistant to disease. The latest attempts involving "attention mechanisms" appear to hold a great deal of promise. It also needs to combine various kinds of information-not only genetic information, but also growing conditions and characteristics of the plants. This will allow the growth of sugarcane that not only resists disease, but also exhibits healthy growth under a broad range of environmental conditions.

Acknowledgments

We are deeply grateful to Mr. Rudi Mai and Mr. Qixue Liang for their invaluable support in data compilation and verification, which significantly contributed to refining our manuscript. We also wish to express our sincere appreciation to the two anonymous reviewers for their thorough evaluation and constructive feedback, which greatly enhanced the quality of this work.

Funding

This study was supported by the Research and Training Fund of the Hainan Institute of Tropical Agricultural Resources (Project No. H2025-03).

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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