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Application of Genome Editing in Sugarcane for Sugar Production: CRISPR/Cas9-Based Precision Improvement of Sugar Accumulation and Stress Tolerance

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Abstract This study explored the application of CRISPR/Cas9 genome editing technology in sugarcane improvement, which increased the sugar content and stress recovery ability of sugarcane. Sugarcane is an important crop for sugar production and bioenergy production. Its productivity and quality are limited by environmental challenges and the complexity of its genetic structure. The polyploid characteristics of sugarcane are facing huge challenges. However, the emergence of CRISPR/Cas9 genome editing technology provides precise tools to address these limitations, improving sugar metabolism and enhancing resistance to various stresses. The integration of multi-omics technologies and the pursuit of transgenic editing techniques have expanded the application scope of genome editing in sugarcane. Integrate multi-omics analysis and emerging gene editing tools in the future precise improvement research of sugarcane, a sugar crop, to promote the improvement of sugarcane varieties and the sustainable development of the industry.
Keywords Sugarcane; CRISPR/Cas9; Genome editing; Sugar accumulation; Stress tolerance

1 Introduction

As one of the major crops worldwide, sugarcane can provide bioenergy and sugar. The wide application of sugarcane in the food and processing industries supports the agricultural economy (Hussin et al., 2022; Krishna et al., 2023). As the global demand for sugar and biofuels continues to grow, the cultivation of sugarcane is becoming increasingly common, and increasing sugar production is also crucial (Krishna et al., 2023).

Farmers are facing huge challenges. Pests, diseases and harsh environmental conditions, such as drought, saline-alkali soil or extreme weather, may significantly affect the yield of sugarcane (Li et al., 2022; Krishna et al., 2023). Furthermore, climate change and new stress factors have exacerbated these problems, leading to a decline in the stability of crops (Li et al., 2022; Krishna et al., 2023). Although traditional breeding methods are helpful, their progress is slow and the improvement results are usually small (Abdelrahman et al., 2018; Muha-Ud-Din et al., 2023).

This study will explore the precise modification of sugarcane genes by CRISPR/Cas9 gene editing technology to cultivate new sugarcane germplasm that stores more sugar and has better resistance. Relying on the high-precision and high-efficiency features of CRISPR/Cas9 technology, it can promote the sustainable development of agricultural production and also solve the regulatory issues related to genetically modified crops. This study will also explore this method that combines modern gene editing technology with traditional breeding. The application of CRISPR/Cas9 gene editing technology is equivalent to injecting “technological impetus” into traditional breeding, accelerating the breeding speed of sugarcane resistant varieties, and improving the breeding efficiency and success rate.

2 Sugar Metabolism and Genetic Characteristics of Sugarcane

2.1 Key mechanisms of sugar accumulation

Sugarcane is one of the main sources of sugar and bioenergy. The core of the economic value of sugarcane lies in the accumulation process of sugar. The accumulation of sugar in sugarcane involves complex metabolic pathways.

Through photosynthesis, the sugar produced by sugarcane is transformed and stored in the stems of sugarcane. The regulatory network of enzymes and transport proteins can promote the transport and storage of sucrose in plants (Oz et al., 2021; Hussin et al., 2022). These mechanisms are highly efficient and can influence the final output of sugar. A key goal of growing sugarcane is to optimize the sugar accumulation process of sugarcane and increase its yield.

The CRISPR/Cas9 gene editing technology can precisely adjust the pathways related to sugar metabolism. By targeting genes related to sucrose production and storage, in sugarcane breeding, researchers utilized CRISPR/Cas9 to enhance the sugar accumulation efficiency in sugarcane, thereby significantly increasing the overall sugar yield and improving other metabolic functions of sugarcane. CRISPR/Cas9 is an important means for improving sugarcane varieties (Hussin et al., 2022; Krishna et al., 2023).

2.2 Major metabolic and regulatory genes

Sugarcane relies on a complex genetic network to control sugar metabolism, with many metabolic and metabolic genes. Some genes produce regulatory proteins that affect the activity levels of metabolic enzymes. Other genes can directly encode metabolic enzymes and participate in the production and decomposition of sucrose. Among these enzymes, sucrose phosphate synthase, sucrose synthase and invertase are particularly important in controlling the sugar content in plants (Zafar et al., 2020; Kumar et al., 2023).

By using the CRISPR/Cas9 technology, researchers can edit the genes of sugarcane more accurately to increase the sugar content. The CRISPR/Cas9 technology alters the levels of regulatory genes and enzymes, making it easier for sugar in sugarcane to deposit. This gene editing method can improve sugarcane varieties and enable sugarcane to produce more sugar under different environmental conditions (Haque et al., 2018; Kumar et al., 2024).

2.3 Challenges of the polyploid genome

In the genome editing of sugarcane, the polyploid characteristics of the genome are a challenge. The genome of sugarcane is composed of multiple sets of chromosomes, and genetic manipulation will be more complex. In traditional breeding and genetic engineering techniques, targeting multiple alleles simultaneously to achieve the desired traits is also a major challenge (Mohan, 2016; Oz et al., 2021).

During the process of sugarcane breeding, the advancement of CRISPR/Cas9 technology has provided more opportunities for the study of sugarcane polyploid genomes. Enhancing the ability of precise gene editing of multiple alleles can improve the genetic traits of sugarcane. To stimulate the potential of genome editing in sugarcane, efficient transformation and screening technologies still need to be developed (Mohan, 2016; Tanveer et al., 2024).

3 The application of CRISPR/Cas9 in Sugarcane

3.1 Gene editing increases sugar production

Researchers used CRISPR/Cas9 to adjust the genes of sugarcane that produce and store sugar. Sugarcane has a tricky genome, and CRISPR/Cas9 technology can perform small and precise edits. By altering key sugar-making genes, plants can produce and retain more sugar in their stems. Some edited types of sugarcane now store additional sugar (Augustine, 2017; Hussin et al., 2022). This proves that gene editing can help farmers cultivate new varieties of sugarcane that are sweeter and stronger.

The CRISPR/Cas9 technology replaces “superior” genes with “inferior” ones to optimize the sugar metabolism process in sugarcane. By using the method of “homologous directed repair (HDR)”, researchers can carry out targeted nucleotide substitution, enabling sugarcane to better utilize its own genes to produce more sugar and improve sugar production efficiency (Oz et al., 2021). Since each gene in sugarcane has many copies, precise editing is of great significance. CRISPR/Cas9 helps improve the genetic combination of sugarcane and increase its sugar yield.

3.2 Edit stress resistance genes

Sugarcane is confronted with numerous troubles such as drought, saline-alkali land, high temperature and pests and diseases. The CRISPR/Cas9 technology can address these challenges by editing genes related to these stresses. Editing genes related to drought resistance helps sugarcane grow better in arid environments, which means more stable sugarcane yields and reduced sugarcane losses (Krishna et al., 2023; Tanveer et al., 2024).

Technology is constantly advancing, and the genetic modification system is also constantly being optimized. Sugarcane can also be grown without transgenic genome editing. Through transient expression systems and precise screening processes, researchers can cultivate gene-edited plants without involving genetically modified organisms. This solved the regulatory problem and laid the foundation for the commercial application of CRISPR/Cas9 technology in sugarcane improvement (Krishna et al., 2023). Integrating genome editing technology into sugarcane breeding is a transformative innovation in this transformation method, which is of great significance and importance.

3.3 Optimization of sugarcane conversion system

The application effect of CRISPR/Cas9 in sugarcane depends on the efficiency of the transformation system. Due to the complexity of the sugarcane genome, it is of great significance to optimize the transformation methods and obtain reliable genome editing results. Strengthening the transformation methods (including agrobacterium-mediated technology and bio-particle delivery methods) to improve the binding of CRISPR/Cas9 components in sugarcane cells (Eid et al., 2021; Laksana et al., 2024). These improvements have increased the success rate of genome editing in sugarcane and cultivated varieties with excellent genes.

With the advancement of technology, the transformation system is constantly being optimized. Sugarcane plants can be grown without transgenic genome editing. Through transient expression systems and precise screening processes, researchers can cultivate gene-edited plants without involving genetically modified organisms. This resolves regulatory issues and lays a solid foundation for the commercial application of CRISPR/Cas9 technology in sugarcane improvement (Krishna et al., 2023). Integrating genome editing technology into sugarcane breeding is a transformative innovation in this transformation method and is of great significance.

4 Gene editing strategies to increase sucrose accumulation in sugarcane

4.1 Editing genes related to sucrose-synthesizing enzymes

The coding gene of sucrose synthase was precisely modified, which increased the sugar accumulation in sugarcane. Using the CRISPR/Cas9 system, researchers can target key genes (sucrose phosphate synthase (SPS); sucrose synthase (SuSy). The CRISPR/Cas9 system regulates the expression of these genes, which can enhance the efficiency of sucrose production and thereby increase sugar output. The successful application on other crops further proves that this method can improve sugarcane varieties (Arora and Narula, 2017; Hussin et al., 2022).

CRISPR/Cas9 simultaneously edits multiple alleles, which can significantly enhance the trait of sugar accumulation. At the same time, for multiple loci, sugarcane varieties with high sucrose content can be cultivated. The accuracy and efficiency of CRISPR/Cas9 technology lay a solid foundation for complex genetic modification and is a sustainable way to increase sugar production (Oz et al., 2021; Tanveer et al., 2024).

4.2 Movement and storage of sucrose in sugarcane

In sugarcane plants, the key to increasing the sugar content of sugarcane lies in the transportation of sugar. The sugar transport process involves the synthesis of sugar in the leaves of sugarcane and its transportation to the stems for storage. The CRISPR/Cas9 technology can optimize the sugar production process by editing the genes that control this process. By modifying these genes with CRISPR/Cas9, sugar can be transported more efficiently, helping sugarcane store more sugar and thereby increasing the total sugar yield (Chen et al., 2019; Krishna et al., 2023). Gene editing can also change the distribution pattern of sugar in sugarcane. Some genes in sugarcane can determine whether sugar is stored or used for other functions in sugarcane growth. Editing these genes can promote more sugar storage rather than consumption in other parts to enhance the sugar accumulation efficiency in sugarcane (Zafar et al., 2020; Ahmar et al., 2023).

4.3 Altering key control genes (transcription factors)

Edit the upstream transcription factors of gene expression related to sugar metabolism to increase sugar accumulation in sugarcane. Researchers used CRISPR/Cas9 technology to precisely edit these genes, improve the functions of multiple genes related to sugar, enhance the overall function of related metabolic pathways, and increase the sugar yield of sugarcane (Abdelrahman et al., 2018; Kumar et al., 2023).

Precise regulation of the expression of transcription factors can optimize the glucose metabolism of sugarcane. Under the influence of climate change or some harsh conditions, sugarcane can still maintain a good yield. The application of CRISPR/Cas9 technology enables researchers to edit these transcription factors more easily and effectively, thereby promoting sugar accumulation in sugarcane and increasing sugarcane yield (Hussin et al., 2022; Tanveer et al., 2024).

5 Improvement of the stress resistance of sugarcane

5.1 Drought-resistant salt gene editing

The CRISPR/Cas9 technology edits the key genes in sugarcane that help resist drought and salinization. Editing the genes that control the number of stomata (small holes on the surface of plants) can help sugarcane retain more water during drought and reduce water loss through transpiration (Kumar et al., 2020; Hussin et al., 2022). CRISPR/Cas9 can also target genes related to salt tolerance, those involved in molecular transfer or stress response signal transmission, enhancing the survival ability of sugarcane in saline-alkali environments (Farhat et al., 2019; Kumar et al., 2023).

CRISPR/Cas9 can knock out harmful genes in sugarcane and precisely edit the genes related to some beneficial traits in sugarcane. For instance, a substance that helps plants cope with environmental stress, controlling osmotic protectants and editing the genes of this substance, can help sugarcane better maintain cellular balance and enhance stress resistance in harsh environments. CRISPR/Cas9 plays a significant role in crops such as rice and can enhance the drought resistance and salt tolerance of these crops (Figure 1) (Kumar et al., 2020; Kumar et al., 2024). Using CRISPR/Cas9 technology, researchers can cultivate sugarcane with greater stress resistance, and this variety of sugarcane can produce sugar stably for a long time.

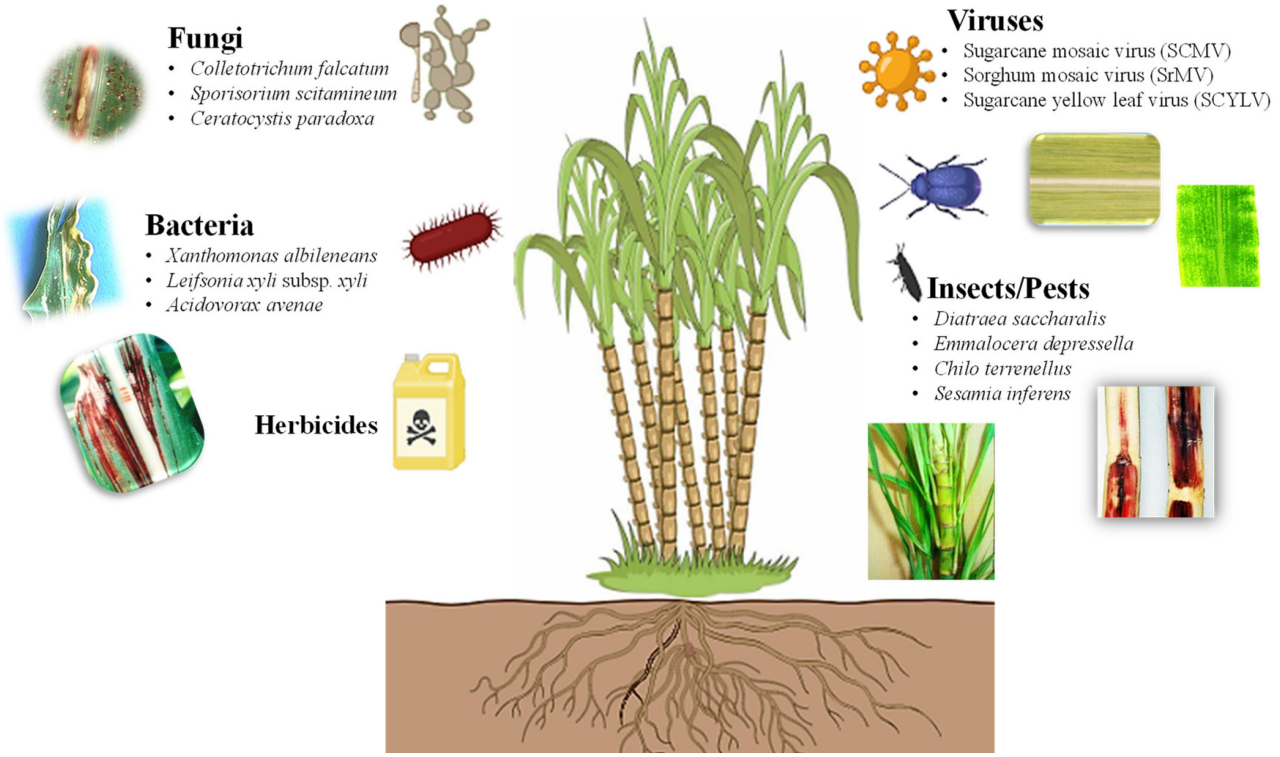

Figure 1 Types of biotic stresses that affect growth, yield, and productivity of sugarcane (Adopted from Kumar et al., 2024)

5.2 Targeting disease resistance genes

Through CRISPR/Cas9 editing, genes that play a key role in pathogen recognition and defense responses can enhance the disease resistance of sugarcane. Editing the resistance (R) gene can enhance the recognition ability of sugarcane against the effector of pathogenic bacteria, thereby strengthening its immune response ability. This method. CRISPR/Cas9 can edit the R gene and is also applicable to other crops, which can endow crops with resistance to various pathogens (Arora and Narula, 2017; Krishna et al., 2023).

Researchers have utilized CRISPR/Cas9 to target the specific gene, the R gene, in sugarcane that is involved in pathogen recognition and defense responses, in order to enhance the susceptibility of sugarcane to diseases. As a result, the frequency of sugarcane being infected with diseases will be reduced, allowing farmers to use less spray and grow healthier sugarcane. This gene editing technology helps control other crops such as beans and rice, enhancing the resistance of these plants to bacteria, fungi and viruses (Wang et al., 2021; Kumar et al., 2024). The precise editing of these genes has made the new sugarcane varieties more stress-resistant and have higher yields.

5.3 Edit the stress response pathways

CRISPR/Cas9 can help sugarcane cope with environmental stress during growth by modifying the genes that control the stress response. These genes, transcription factors and kinases play an important role in the process by which plants cope with adverse conditions. For example, by editing the genes in the ABA signaling pathway, CRISPR/Cas9 can enhance the water-saving capacity of sugarcane, thereby enhancing the drought resistance of sugarcane (Farhat et al., 2019; Ahmar et al., 2023). Similarly, the genes in the salicylic acid (SA) and jasmonic acid (JA) pathways were edited to enhance the disease and pest resistance of sugarcane (Kumar et al., 2023).

CRISPR/Cas9 can simultaneously edit multiple genes and is also more likely to regulate complex stress response systems. Sugarcane is polyploid, and each gene in sugarcane can have multiple copies. CRISPR can target multiple genes simultaneously, which is particularly beneficial for sugarcane. CRISPR/Cas9 modifying different genes of the same pathway can more effectively enhance the stress resistance of sugarcane, enabling it to grow stronger and have a higher yield under harsh conditions, and also laying the foundation for cultivating better sugarcane varieties (Hussin et al., 2022; Mir et al., 2022).

6 Challenges and Future Perspectives

6.1 Complexity and off-target issues of the sugarcane genome

The genome of sugarcane is very complex because it is polyploid. Polyploid leads to multiple copies of genes, so it is difficult to achieve targeted modifications without affecting the copies of other genes, and it may accidentally change the wrong part of the DNA. When using CRISPR/Cas9, it may also affect areas that should not be changed. These unwanted changes may bring about new problems or reduce the ability of plants to adapt to the environment (Mohan, 2016; Oz et al., 2021; Hussin et al., 2022).

Researchers are also striving to improve the CRISPR/Cas9 system, studying a newer and more precise version of the Cas9 enzyme and designing better guide RNA to help the system locate the correct position (Oz et al., 2021; Hussin et al., 2022). Combining multi-omics tools such as genomics, transcriptomics and proteomics can help researchers better understand the genome of pitaya, which is conducive to selecting better target genes and conducting more precise gene editing (Figure 2) (Oz et al., 2021; Hussin et al., 2022).

6.2 Management challenges and public acceptance

Sugarcane is one of the genome-edited crops. The main challenge in genome-edited crops is the relevant laws and regulations. Many countries have strict management regulations on genetically modified organisms (Gmos), and genome-edited plants usually also face scrutiny. The presence of genome editing in plants has raised concerns among regulatory agencies, leading to restrictions or delays in the application of some technologies in agriculture (Krishna et al., 2023; Kumar et al., 2024). Some genome editing methods, such as ribonucleoprotein technology, do not introduce exogenous DNA into plants, which can reduce some regulatory challenges (Arora and Narula, 2017; Krishna et al., 2023).

Public understanding and acceptance of genome-edited crops are equally important. Due to concerns over safety and ethical issues, the public is skeptical of genetically modified crops. It is of great significance for regulatory agencies to explain to the public the safety and benefits of genome editing technology and gain public trust (Arora and Narula, 2017; Kumar et al., 2024). Involving farmers, consumers and policymakers in discussions, the public can understand the potential of genome editing, which can increase sugarcane yields and thus make these genome-edited crops more socially acceptable (Arora and Narula, 2017; Kumar et al., 2024).

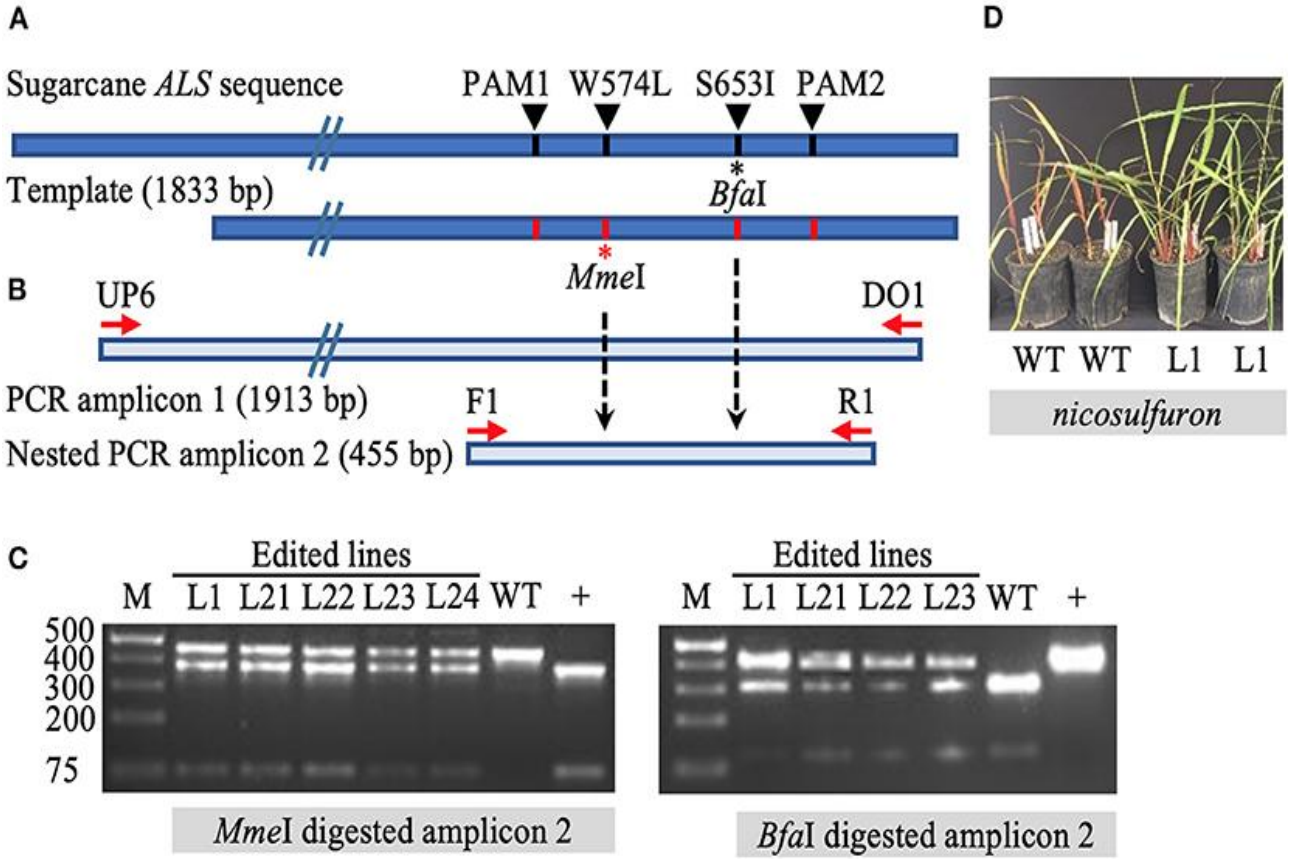

Figure 2 Identification of gene targeting in sugarcane by restriction endonuclease assays and herbicide application (Adopted from Oz et al., 2021)

Image caption: (A) Repair template compared to sugarcane acetolactate synthase (ALS) gene. The 1 833 bp template carries four nucleotide substitutions, one at the codon positions W574L (introducing the MmeI recognition site) and S653I (eliminating BfaI recognition site), as well as two modified PAM sites (PAM1 and PAM2) (preventing template cleavage by Cas9 complexed with sgRNA1 or sgRNA2). Amino acid residue numbering in the sugarcane ALS gene follows the Arabidopsis nomenclature. (B) Schematic representation of primer annealing positions. Primers DO1 and UP6 were used to amplify a 1,913 bp fragment. Using primer UP6 prevented amplification of randomly inserted template by annealing outside the repair template. Primers F1 and R1 were used to generate a 455 bp nested PCR amplicon for analyses by restriction enzyme digestion. (C) Restriction-digestion pattern of nested PCR amplicons from wild-type (WT) and edited lines after digestion with MmeI or BfaI. + indicates positive control that includes the MmeI site and lacks the BfaI site. (D) Vegetatively propagated edited line L1 with multi allele conversion of W574L and S653I was tolerant to application of the herbicide nicosulfuron (Accent® DuPont) at 95 g ha^{-1}, in contrast to non-edited WT plants (Adopted from Oz et al., 2021)

6.3 Emerging tools and multi-omics integration

Genome editing technology is developing rapidly. New tools such as base editing and start editing are integrated with CRISPR technology. These new technologies can directly modify genes without cutting the DNA strand, thereby enhancing the safety and accuracy of crop gene editing. Because the genome of sugarcane is very complex, the advancements of these technologies are of great significance to sugarcane. Using these new tools, researchers can make smaller and more precise alterations to the DNA of plants (Arora and Narula, 2017; Haque et al., 2018).

By bringing in multi-omics data- including gene sequences, RNA activity, and protein levels- researchers can better understand how sugarcane works. This helps them choose the best targets for editing. Combining these tools may lead to better sugarcane varieties that grow well and produce more under different conditions.

7 Concluding Remarks

CRISPR/Cas9 is an important tool in sugarcane breeding. The genome of sugarcane is extremely large and complex. CRISPR/Cas9 can precisely and on a small scale edit specific genes of sugarcane or edit these genes in a controllable way, thereby helping to cultivate new sugarcane varieties more quickly. These newly cultivated varieties usually have a higher sugar content, while reducing unnecessary genetic changes and risks in sugarcane. They can better adapt to extreme climates or poor soil, making the breeding process of sugarcane more efficient and stable.

The use of CRISPR/Cas9 helps sugarcane grow better and achieve good yields even under harsh conditions. By editing genes related to yield and tolerance with CRISPR/Cas9, researchers can cultivate sugarcane varieties that still grow well in drought, pest attacks or saline-alkali land, which is important for maintaining stable sugar production and helping to produce biofuels. CRISPR/Cas9 endows sugarcane with stronger natural defense capabilities and is the key to coping with the constantly changing environment.

In sugarcane breeding, the use of CRISPR/Cas9 can ensure better development of sustainable agriculture, reduce the demand for chemical fertilizers and pesticides, make the plants themselves stronger, and also be beneficial to environmental protection. The new gene editing method does not add exogenous DNA. These crops may not be classified as genetically modified organisms, meaning that these genetically modified organisms can be approved more quickly and be more easily sold and accepted in markets around the world.

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Conflict of Interest Disclosure

The author affirms 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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