Krishan K. Verma · Xiu-Peng Song ·
Dong-Liang Huang ·
Renato de Mello Prado ·
Yang-Rui Li *Editors*

Revolutionizing Sugarcane Molecular Breeding and Biotechnological Approaches

Current Status and Future Strategies

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Preface

Sugarcane, a major C_4 crop, is extensively cultivated in arid and semiarid zones globally, providing valuable raw materials for the production of sugar, bioethanol, and various other by-products. Sugarcane breeding has made a major contribution to the expansion and profitability of sugar agroindustry around the globe. Nowadays, sugarcane breeding programs are ongoing in the majority of major cane-producing countries. Advancements in genome-wide selection (GWS) studies and molecular breeding techniques have unlocked new opportunities to harness the natural genetic variations that contribute to drought resistance in sugarcane. Renewable plant-based fuels may offer a viable substitute for reducing nonrenewable energy sources. Producing biofuel has a variety of environmental benefits, some of which include downregulating the release of greenhouse gas emission and minimizing their contribution to global warming. The main objective of the sugarcane breeding program is to release improved cane varieties that solve challenges affecting cane farming and production. Released sugarcane varieties should outperform existing ones in terms of sugar yield and climate resilience. The main objective to select varieties that can produce more profit to the sugar industries and the farmers.

New sugarcane varieties are developed by interspecific and intergeneric hybridization between different species of sugarcane, including *Saccharum officinarum, Saccharum spontaneum*, and other related plant varieties. Sugarcane breeding programs are mainly divided into germplasm collection and trait evaluation, genetic improvement and expansion of gene pool, and variety development. The extensive breeding process requires at least 12–14 years. The precise phenotyping in sugarcane is very complex due to lodging and suckering caused by agroclimatic variables and farming practices. This type of phenotyping requires data from the plant and ratoon crop experiments carried out across locations and seasons. Considerable advancements have been made in genetic research of major agronomic characteristics.

The analysis of genetic diversity by genome-wide markers assists in the formation of core set germplasm and represents the total genomic diversity in the *Saccharum* gene bank. The genome-wide association (GWS) studies and genomic prediction in the *Saccharum* gene bank are useful to assess the complete genomic

resources for cane yield, commercial cane sugar, tolerance adverse environmental factors, and other agronomic characteristics. The implementation of genomic selection in pre-breeding, genetic base-broadening programs helps in precise introgression of specific genes, and recurrent selection programs enhance the optimum frequency of suitable alleles in the population with a considerable loss in breeding cycles and the size of population. The integration of environmental covariates and genomic prediction in multi-environment demonstrations helps in the prediction of varietal performance efficiency for different agro-climatic zones.

The proposed book discussed its focus on increasing the genetic gain over time, cost, and resource allocation at different stages of breeding programs in sugarcane. Advance technological developments indicate the potential to enhance our understanding of the sugarcane plants by emerging genomic technologies. It provides updated information on various advance applications that are employed for sugarcane improvement via molecular breeding and biotechnological strategies and will be more interest to plant scientists, biotechnologists, molecular biologists, and breeders who work on sugarcane crop.

We hope that this book will serve as a valuable reference book for sugarcane molecular breeding and biotechnological approaches and facilitate an exchange of recent information and ideas about the current status and development of sugarcane molecular breeding and biotechnological innovations to harness the significance of sugar and bioenergy crop.

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Chapter 1
Climate Change and Agrotechnological Strategies for Sugarcane Breeding

Mohseen Riaz Ud Dean

Abstract The Fiji sugar industry stands at a crossroads, confronted by the dual challenges of pathogen pollution and climate change, alongside an urgent need for technological modernization. Yet, within these challenges lies a unique opportunity to transform the sector into a model of resilience and sustainability. Pathogen pollution poses a significant threat to sugarcane yields and quality, but advancements in biotechnology, including marker-assisted selection (MAS) and genetic modification, offer promising solutions. These techniques can fast-track the development of disease-resistant sugarcane varieties, reducing reliance on chemical inputs and enhancing crop resilience. Simultaneously, the impacts of climate change necessitate the breeding of climate-resilient varieties and the adoption of integrated pest and disease management (IPDM) practices. These efforts are vital to maintaining productivity due to increasing environmental variability. Agrotechnological advancements such as precision agriculture, automation, and integrating Internet of Things (IoT) technologies present transformative opportunities. These innovations can improve efficiency, reduce resource use, and bolster the industry's resilience against climatic and economic uncertainties. However, the success of these strategies hinges on a collaborative approach that brings together policymakers, industry stakeholders, researchers, and international partners. Fostering an enabling environment for innovation through strategic policy interventions and capacity building initiatives is crucial. By investing in developing skills and infrastructure, Fiji can position its sugarcane industry as a sustainable and technologically advanced agriculture leader. Access to international funding and expertise will further enhance the sector's capacity to adopt cutting-edge technologies and practices. The future of Fiji's sugar industry depends on its ability to adapt and embrace change. A commitment to sustainable development and resilience will ensure that this sector plays a vital role in the nation's economy and heritage. By leveraging agrotechnological advancements and fostering global collaborations, Fiji can safeguard its sugarcane industry and set a benchmark for resilient agricultural practices worldwide. This

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chapter will explore the potential pathways for revitalizing Fiji's sugarcane industry, emphasizing the importance of innovation, strategic partnerships, and sustainable practices.

Keywords Agrotechnology · Climatic variables · Fiji · Pathogenic diseases · Policy · Sugarcane breeding

1.1 Introduction

Sugarcane production is a cornerstone of Fiji's economy, providing employment to a significant portion of the population and contributing substantially to the nation's GDP through sugar exports (Dean 2019). As with many other agricultural sectors worldwide, Fiji's sugarcane industry faces escalating threats from multiple fronts, including pathogen pollution and climate change, subjecting the industry to the need for sustainable agrotechnological advancements (Dean 2022). These challenges affect crop yields and have far-reaching implications for the nation's rural livelihoods, food security, and economic stability (Chen et al. 2020; Li et al. 2024a, b). Pathogen pollution has become a persistent issue in sugarcane farming globally (Song et al. 2019; Huang et al. 2024; Lahlali et al. 2024), with diseases such as smut and leaf scald presenting significant risks to productivity and crop quality (Singh et al. 2019). To this end, pathogen-resistant breeding programs have achieved varying levels of success globally, but Fiji's context requires tailored solutions due to its unique climatic conditions and disease pressures. To address these challenges effectively, insights from other major sugarcane-producing nations can provide valuable lessons (Malviya et al. 2020, 2022). For example, Australia's approach to smut-resistant varieties (Bhuiyan et al. 2021) offers potential pathways for Fiji to enhance its breeding programs.

Alongside pathogen threats, the impacts of climate change are becoming increasingly severe (Nand et al. 2023; Bhatt et al. 2023a, b; Jagadesh et al. 2024). Rising temperatures, erratic rainfall, and frequent extreme weather events, such as cyclones, put immense pressure on sugarcane cultivation (Verma et al. 2019a, b; Prakash 2021). These environmental stressors can exacerbate disease spread, reduce crop resilience, and threaten yield quality (Singh et al. 2020, 2022; Verma et al. 2020a, 2024a). Solutions to these challenges must be forward-thinking, mitigating climate impacts and increasing crop resilience by developing climate-adaptive sugarcane varieties (Naidu et al. 2017; Sharma et al. 2022; Afghan et al. 2024).

In the face of these dual pressures, sustainable technological innovation offers significant promise (Padhiary et al. 2024; Singh et al. 2021a, b; Wu et al. 2022; Liang et al. 2024). For example, genetic engineering, precision agriculture, and mechanization advances can help sugarcane farmers in Fiji achieve higher productivity and sustainability. By drawing on successful agrotechnological strategies from across the globe, Fiji can explore ways to boost its sugarcane sector's resilience. These global insights can inform the development of integrated approaches

that combine pathogen resistance, climate adaptation (Kumari et al. 2022, 2023; Xu et al. 2024; Verma et al. 2023a, b), and technological innovation, paving the way for a more sustainable sugarcane industry (Verma and Singh 2023; Solanki et al. 2023).

This chapter examines the challenges of pathogen pollution and climate change and explores examples of globally advanced sustainable agrotechnological interventions in sugarcane breeding. This chapter, whilst focusing on Fiji's sugar industry and drawing from international examples, will provide a comprehensive analysis of potential pathways to improve the resilience and productivity of Fiji's sugarcane sector. The aim is to offer valuable insights for policymakers, industry leaders, and researchers seeking to address these complex and interconnected systemic issues in Fiji's sugarcane sector.

1.2 An Overview of Pathogen Pollution in Sugarcane

Pathogen pollution poses a significant threat to sugarcane cultivation worldwide, and this is especially true for countries like Fiji, where the sugarcane industry plays a pivotal role in the economy. Pathogen pollution refers to introducing and spreading harmful pathogens—such as bacteria, fungi, and viruses—within crops, leading to reduced yields, compromised quality, and, in severe cases, crop failure (Anas et al. 2021a, b; Huang et al. 2022; Msomba et al. 2024). In Fiji, sugarcane diseases such as sugarcane smut (*Sporisorium scitamineum*), leaf scald (*Xanthomonas albilineans*), and sugarcane mosaic virus (SCMV) are particularly prevalent (Smith and Velde 1994; Dean 2019; Malviya et al. 2021, 2024). These diseases impair plant growth and reduce sucrose content, thus directly affecting the economic value of the crop (Song et al. 2021, 2022; Bertasello et al. 2023). While sugarcane breeders have made strides in developing disease-resistant varieties, the evolving nature of these pathogens continues to pose ongoing challenges (Singh et al. 2021; Krishna et al. 2022; Zheng et al. 2023).

1.2.1 Sugarcane Smut Disease

One of Fiji's most persistent diseases affecting sugarcane fields is smut, caused by the fungus *Sporisorium scitamineum*. Smut is highly contagious and can lead to yield losses of up to 40%, manifesting in black, whip-like structures on the plants (Dean 2019). The pathogen spreads through infected soil, water, and plant material, rapidly affecting entire fields if not properly managed. The Australian sugar industry faced a similar crisis in the late 1990s and early 2000s when smut spread uncontrollably (Croft and Braithwaite 2006). However, through a combination of strict quarantine measures and the development of smut-resistant varieties, the country was able to control the disease (Croft et al. 2000). Australia's experience offers

valuable insights for Fiji, where implementing similar strategies could curb the spread and impact of smut on sugarcane crops.

1.2.2 Leaf Scald Disease

Leaf scald, caused by the *Xanthomonas albilineans* bacterium, is another severe disease impacting Fijian sugarcane. Characterized by leaf necrosis, reduced sucrose levels, and eventual plant death, leaf scald spreads through contaminated tools and machinery (Dean 2019; Chen et al. 2023, 2024). This highlights the importance of maintaining stringent sanitation practices during sugarcane cultivation. In Louisiana, USA, an integrated approach combining resistant varieties and strict sanitation has successfully reduced leaf scald incidence, offering a potential model for Fiji's sugarcane industry (Padgett et al. 2022; Tian et al. 2023; Bhatt et al. 2024). Adopting similar practices could help mitigate the risks posed by this devastating pathogen.

1.2.3 Sugarcane Mosaic Virus (SCMV)

Sugarcane mosaic virus (SCMV), a viral disease, is also a pressing concern for sugarcane growers in Fiji (Daugrois et al. 2023). Transmitted by aphids, SCMV affects plant vigor and sucrose content, leading to substantial yield losses. Countries like Brazil have successfully managed SCMV by integrating resistant sugarcane varieties with pest control measures aimed at aphid populations (Li et al. 2024a, b). For Fiji, an integrated pest management (IPM) approach—combining the cultivation of resistant varieties with environmentally sustainable pest control methods—could significantly reduce the impact of SCMV on its sugarcane industry.

1.3 Lessons from Global Pathogen Management Strategies

Around the world, sugarcane-growing nations have developed innovative approaches to manage the effects of pathogen pollution. India and Brazil, in particular, have made notable progress in breeding pathogen-resistant varieties. In India, for instance, the fight against red rot, a devastating fungal disease, has led to the development of resistant varieties through traditional breeding methods and molecular techniques (Hossain et al. 2020; Sharma et al. 2021; Guo et al. 2023). These advances highlight the importance of integrating conventional and modern breeding approaches to address evolving pathogen challenges. Brazil, a leading producer of sugarcane, has embraced genetic engineering to combat pathogen pollution. By incorporating disease-resistant genes into sugarcane cultivars, Brazil has developed varieties more resilient to fungal and viral infections (Cursi et al. 2022). This use of

genetic engineering not only helps reduce yield losses but also minimizes the reliance on chemical pesticides, leading to environmental and economic benefits. For Fiji, adopting similar advanced breeding techniques could strengthen the resilience of its sugarcane industry and promote sustainable farming practices.

1.4 Future Directions: Solutions to Pathogen Problems in Fiji

1.4.1 Biotechnology and Marker-Assisted Selection (MAS)

The application of biotechnology in sugarcane breeding is advancing rapidly, offering promising solutions for developing pathogen-resistant varieties. In Australia, researchers have employed marker-assisted selection (MAS) to identify the genetic markers associated with disease resistance in sugarcane, particularly for rust and smut (Sun et al. 2019; Deng et al. 2023). MAS significantly reduces the time required to develop new varieties by enabling breeders to select plants with desirable traits early in the breeding process. For Fiji, investing in MAS technology could accelerate the breeding of sugarcane varieties resistant to prevalent diseases such as smut, leaf scald, and SCMV. By adopting this approach, Fijian breeders can streamline the process of developing resilient sugarcane crops, ensuring that the industry remains competitive in the global market.

1.4.2 Pathogen Resistance Through Genetic Engineering

Genetic engineering offers another avenue for combating pathogen pollution in sugarcane. Unlike traditional breeding methods, which are labor-intensive and time-consuming due to sugarcane's complex polyploidy and long growth cycle, genetic engineering allows breeders to directly introduce disease-resistant genes into sugarcane cultivars (Hoang et al. 2015). This can significantly accelerate the breeding process and enhance the crop's resilience to various pathogens. Brazil's success with genetically modified (GM) sugarcane varieties, which demonstrate resistance to both fungal and viral pathogens, underscores the potential of this technology. By adopting GM sugarcane, Brazil has reduced pesticide use, lowered production costs, and minimized environmental impact (Cheavegatti-Gianotto et al. 2011; Verma et al. 2024b, c). Fiji could benefit from similar advancements, although introducing GM crops requires careful consideration of regulatory frameworks and public acceptance. Transparent communication and public consultations would be essential to ensure that GM technology is embraced as part of Fiji's agricultural strategy (Bacolod 2014), as different thoughts of schools in favor of and against it emerge and scientific validations continue to nurture (Ghimire et al. 2023).

1.4.3 Integration of Global Pathogen Management Strategies into Fiji's Breeding Programs

To enhance the resilience of its sugarcane industry, Fiji could integrate global pathogen management strategies into its breeding programs. Collaborating with international research institutions such as Australia's Sugar Research Australia (SRA) and Brazil's Centro de Tecnologia Canavieira (CTC) could provide Fijian breeders access to resistant varieties and advanced diagnostic tools. These partnerships could also facilitate knowledge transfer, enabling Fiji to leverage cutting-edge technologies such as genomic sequencing to detect and characterize pathogens more efficiently (Meena et al. 2022; Li et al. 2023; Singh et al. 2023). Moreover, establishing a central pathogen resistance database could enhance collaboration among Pacific nations facing similar challenges. Such a database would enable researchers to share data on pathogen prevalence, resistance traits, and breeding outcomes, fostering regional cooperation in disease management. Additionally, Fiji could adopt Australia's rigorous quarantine and biosecurity measures, which include pathogen testing for imported varieties and routine surveillance of sugarcane fields to detect outbreaks early (McMaugh 2005; Joshi et al. 2023).

In summary, pathogen pollution remains a formidable challenge for sugarcane breeders, particularly in regions like Fiji, where the sugarcane industry is vital to the economy. However, by adopting global best practices in pathogen management—such as disease-resistant breeding, integrated pest management, and biotechnology—Fiji can build a more resilient sugarcane industry. Collaboration with international research institutions, investment in modern breeding technologies like MAS and genetic engineering, and establishment of robust quarantine measures will be vital to ensuring the long-term sustainability of Fiji's sugarcane sector. As the industry faces evolving pathogen threats, these strategies offer a clear pathway to safeguarding crop yields and farmers' livelihoods dependent on sugarcane cultivation.

1.5 The Impact of Climate Change on Sugarcane Cultivation

Climate change presents profound and growing challenges to the sustainability of sugarcane cultivation globally (Verma et al. 2022a, 2023c, d), particularly in tropical regions such as Fiji. As a susceptible crop to environmental conditions, sugarcane's growth, yield, and quality are directly affected by temperature, rainfall, and the increasing frequency of extreme weather events. In recent years, Fiji has witnessed significant disruptions to its sugarcane farming due to erratic weather patterns, including prolonged droughts, excessive rainfall, and the intensification of tropical cyclones (Guo et al. 2021; Nand 2023). These climatic changes threaten both the crop's productivity and the livelihoods of the farmers who depend on it.

1.5.1 Climate Change Challenges in Sugarcane Cultivation

One of the most pressing impacts of climate change on sugarcane is the variability in temperature and rainfall. Sugarcane requires a precise range of temperatures to optimize photosynthesis and sugar accumulation (Verma et al. 2020b, c, 2024d, e). As global temperatures rise, excessive heat stress can reduce sucrose content, a critical factor determining the crop's economic value. High temperatures can also affect the flowering and seed-setting stages, ultimately decreasing yield (Xu et al. 2022; Joshi et al. 2022). Moreover, the unpredictability of rainfall disrupts soil moisture levels, which are vital during crucial sugarcane growth stages. Water stress, particularly during critical phases such as germination and tillering, can result in stunted growth and lower biomass accumulation (Zhao and Li 2015; Guo et al. 2022; Verma et al. 2020d, e, f). Fiji's situation is not unique, and lessons can be drawn from other sugarcane-producing nations. For instance, in Brazil—one of the world's largest sugarcane producers—researchers have developed climate-resilient varieties that are more tolerant to drought. These varieties, often crossbred with drought-resistant wild relatives of sugarcane, offer a potential solution for countries like Fiji. However, breeding for climate resilience is a complex endeavor, requiring the integration of multiple traits such as heat tolerance, water-use efficiency, and pest resistance. These traits must be combined to allow sugarcane varieties to thrive in increasingly unpredictable climates (Dlamini 2016; Oliveira et al. 2022; Verma et al. 2021a, b, 2022b).

Fiji is particularly vulnerable to tropical cyclones, which have increased in frequency and intensity due to climate change. Cyclones can devastate sugarcane fields, causing physical crop damage and significantly reducing yields. A stark example occurred in 2016 when Cyclone Winston—a Category 5 cyclone—caused widespread destruction and substantial losses in the sugarcane sector. This event underscored the urgent need for cyclone-resilient sugarcane crop varieties and enhanced disaster management strategies to safeguard the industry against future extreme weather events (Dean 2019).

1.6 Future Directions: Solutions to the Impacts of Climate Change on Sugarcane cultivation in Fiji

1.6.1 Breeding Climate-Resilient Sugarcane Varieties

Developing climate-resilient sugarcane varieties has become a critical priority in response to the growing threat of climate change. Breeding for resilience requires selecting traits that enable sugarcane to withstand harsh environmental conditions. These traits include drought tolerance, heat resilience, and rapid regrowth capacity, all essential for sugarcane's survival in extreme weather events. For example, researchers in Australia have successfully bred sugarcane varieties with enhanced

water-use efficiency and salt tolerance. These traits are particularly relevant to Fiji, where rising sea levels and saltwater intrusion pose additional challenges to sugarcane cultivation (Prakash 2021).

One promising approach to breeding climate-resilient sugarcane involves the use of genetic engineering. Advances in biotechnology, particularly tools such as clustered regularly interspaced short palindromic repeats (CRISPR) gene editing, offer new opportunities for enhancing the crop's ability to cope with climate stress (Verma et al. 2021c, d, 2022c). By precisely modifying the genes associated with stress responses, researchers can develop sugarcane varieties more resistant to drought and heat. Notably, this method allows for targeted changes without introducing foreign DNA, making it a more acceptable option in regions where genetically modified organisms (GMOs) face regulatory or public scrutiny (Ahmad and Mukhtar 2017; Aziz et al. 2022). However, adopting genetic engineering in Fiji would require careful consideration of regulatory, ethical, and social factors. While biotechnology offers considerable potential for improving the climate resilience of sugarcane, the introduction of such technology must be managed to ensure public trust and address concerns about environmental and health impacts (Verma et al. 2021e, f). Transparent communication and inclusive policymaking will ensure that genetic engineering can be responsibly integrated into Fiji's sugarcane breeding programs.

1.6.2 Climate Adaptation Strategies: Lessons from Global Practices

Globally, sugarcane-producing countries have employed various climate adaptation strategies (Verma et al. 2022d, e), which offer valuable lessons for Fiji. In India, for instance, large-scale water management systems have been implemented to mitigate the effects of drought on sugarcane production. These systems include the widespread adoption of drip irrigation and rainwater harvesting techniques, which help conserve water and ensure a consistent supply during dry periods. Such practices have significantly enhanced the drought resilience of India's sugarcane industry and could be adapted to Fiji's specific conditions with appropriate modifications (Mohan et al. 2024).

In addition to water management, integrated pest and disease management (IPDM) practices resulting from climate deviations have been widely adopted in countries like Thailand to address climate-induced pest pressures. As climate changes alter the behavior and prevalence of pests, IPDM strategies that combine biological control methods, habitat management, and pest-resistant varieties offer a holistic solution. In Fiji, climate change is expected to exacerbate pest problems, making it imperative for the sugarcane industry to adopt similar approaches to manage the increased risk of pest outbreaks (Nand 2023; Dean 2022). Moreover, integrating pest and disease resistance into sugarcane breeding programs can play a

critical role in climate adaptation. By incorporating resistance to pests and diseases that are likely to thrive under changing climatic conditions, breeders can develop varieties that are not only climate-resilient but also more capable of withstanding the heightened biological threats posed by global warming.

1.6.3 Toward Climate-Resilient Sugarcane Industry

The sustainability of sugarcane cultivation in the face of climate change depends on a multifaceted approach that combines scientific innovation with practical adaptation strategies. For Fiji, this means investing in breeding programs prioritizing climate resilience, drawing on the latest advances in biotechnology and genetic engineering, and incorporating lessons from global best practices. Collaborative efforts with international research institutions and producers will ensure that Fiji's sugarcane industry is equipped to meet the challenges of a changing climate. One stronger candidate is the application of agroecological principles in the cultivation and management of sugarcane farms in Fiji (Dean 2019).

Furthermore, the development of climate-resilient varieties must be complemented by adopting adaptive farming practices, such as water management systems and integrated pest control, to mitigate the impacts of climate change (Verma et al. 2022f, g). By combining these strategies with proactive disaster management plans and community engagement, Fiji can build a sugarcane industry that is more resilient to climate shocks and capable of sustaining rural livelihoods and contributing to national economic growth. In summary, climate change represents both a challenge and an opportunity for the sugarcane industry, specifically sugarcane growers. By embracing innovative breeding techniques and climate adaptation strategies, Fiji can transform its sugarcane sector into a sustainable, resilient, and economic stability model in the face of global environmental change.

1.7 Agrotechnological Strategies in Sugarcane Breeding: A Pathway to Sustainable Advancement

As global agriculture shifts toward sustainability, applying advanced technology in sugarcane breeding offers unprecedented potential to boost productivity and resilience in this significant crop. For countries like Fiji, where sugarcane cultivation is both economically and culturally significant, adopting agrotechnological strategies presents a path to overcoming the challenges posed by climate change, resource constraints, and market demands. This section explores critical agrotechnological innovations such as precision agriculture, biotechnology, mechanization, and digital agriculture—that are transforming sugarcane breeding and cultivation, offering Fiji a sustainable and resilient sugarcane industry for the future.

1.7.1 Precision Agriculture: Enhancing Efficiency in Sugarcane Cultivation

Precision agriculture represents a shift toward highly optimized farming, where advanced technology is used to manage resources like water, fertilizers, and pesticides with remarkable accuracy. In sugarcane breeding, precision agriculture allows for the monitoring and managing of crop health in specific environmental conditions, thereby optimizing input use and promoting sustainability. One crucial component of precision agriculture is soil and crop monitoring. Modern remote sensing technologies like drones and satellite imagery enable real-time soil moisture, nutrient level, and plant health tracking. Brazil, China, the United States, and other countries are considered the leading innovators in precision agriculture for sugarcane and have leveraged these technologies to allow farmers make data-driven decisions that improve both yield and resource efficiency (Wang et al. 2024). For Fiji, where sugarcane productivity varies due to erratic rainfall and uneven soil fertility, adopting soil and crop monitoring tools could enhance irrigation schedules, minimize fertilizer waste, and protect the soil from degradation, ultimately boosting sustainability in sugarcane farming.

Another tool central to precision agriculture is variable rate technology (VRT), which enables farmers to adjust input levels according to the specific needs of different field parts. For instance, Indian sugarcane farmers use VRT to apply fertilizers and pesticides selectively, reducing environmental impact and cutting costs (Balsundram et al. 2023). For Fiji, adopting VRT could address variations in soil fertility and water availability across different regions, efficiently using resources in sugarcane farming while reducing environmental strain.

1.7.2 Biotechnology in Sugarcane Breeding: Advancing Genetic Potential

Biotechnology brings transformative possibilities to sugarcane breeding by providing tools such as genetic modification, molecular markers, and gene editing. These technologies can help accelerate the development of resilient varieties with enhanced traits like disease resistance, drought tolerance, and improved sugar content. Genetic modification and CRISPR gene editing are powerful tools that allow researchers to incorporate desirable traits into sugarcane varieties directly. In Brazil, transgenic sugarcane varieties have been developed to withstand significant pests and pathogens, showcasing the potential of genetic modification in crop resilience (Prado et al. 2024). CRISPR gene editing provides even more precise modifications by enabling scientists to alter specific genes related to stress tolerance without

introducing foreign DNA. This approach could increase public acceptance of genetically enhanced crops, as it aligns with regulatory standards that emphasize natural genetic alterations over transgenic modifications (Shew et al. 2018).

For Fiji, biotechnological advancements could revolutionize sugarcane breeding, particularly in addressing traits like drought and pest resistance that are challenging to achieve through traditional breeding. However, implementing genetic modification and CRISPR technologies would require regulatory reforms and robust public engagement, especially among the growers of sugarcane crops, to address potential environmental and health concerns related to GM crops (McQualter et al. 2004; Kumar et al. 2004). As already mentioned, marker-assisted selection (MAS) is another promising biotechnological tool that enhances breeding efficiency. MAS uses genetic markers linked to desirable traits, such as high sucrose content or pathogen resistance, allowing breeders to select parent plants with increased precision (Sandhu et al. 2022). This technique has been successfully used in Australia's sugarcane breeding programs, helping develop varieties resistant to smut and rust diseases. Integrating MAS into Fiji's breeding initiatives could fast-track the development of resilient sugarcane varieties better equipped to withstand both pathogen pressures and climate variability.

1.7.3 Automation and Mechanization: Increasing Productivity and Reducing Labor Dependence

Mechanization and automation can potentially modernize Fiji's sugarcane industry, especially given the rising labor and production costs. Countries like Brazil and Australia have demonstrated substantial gains in efficiency through mechanized planting, harvesting, and processing, which reduce dependence on labor and improve productivity. Automated harvesting systems, such as mechanical harvesters, reshape the sugarcane industry by streamlining operations and minimizing labor costs. In Brazil, the adoption of mechanical harvesters has lowered labor expenses and reduced crop losses, thus increasing overall yields (Narimoto et al. 2013). In Fiji, where labor shortages and high harvesting costs limit profitability, adopting similar automated systems could boost the efficiency of sugarcane cultivation and help the sector remain competitive.

The Internet of Things (IoT) also plays a critical role in agriculture by enabling interconnected systems that remotely monitor and control farming operations. In India, IoT-enabled sensors track vital parameters like soil moisture, pest populations, and crop health, allowing for real-time adjustments to farming practices (Shahab et al. 2024). For Fiji, implementing IoT applications in sugarcane fields could provide valuable data insights, enabling farmers to make informed decisions on resource management, pest control, and crop monitoring.

1.7.4 Leveraging Agrotechnological Strategies for Sustainable Sugarcane Breeding in Fiji

While agrotechnological advancements hold tremendous potential, implementing these innovations in Fiji requires strategic planning and investment. Challenges such as limited infrastructure, gaps in capacity, and lack of access to funding must be addressed to realize these technologies' benefits fully. Collaborating with international partners is a crucial strategy for overcoming these barriers, as demonstrated by successful technology transfer initiatives in other sugarcane-producing regions, such as the Caribbean, Africa, and Asia. Training and capacity building are essential for equipping Fiji's agricultural workers with the skills to operate advanced equipment and utilize precision agriculture tools. Collaboration with countries like Australia, where expertise in mechanized sugarcane cultivation is well established, could facilitate knowledge transfer and help Fiji's workforce adapt to these technological advancements (Dean 2019). Fiji can lay a strong foundation for a technologically advanced and sustainable sugarcane industry by prioritizing human capital development.

Access to funding and investment in innovation are equally important. Implementing agrotechnological solutions often requires significant investment in both infrastructure and equipment. Policymakers in Fiji could explore funding opportunities through international development agencies and climate finance mechanisms. Organizations such as the Global Environment Facility (GEF) and the Green Climate Fund (GCF) have funded agricultural modernization projects in other developing nations, supporting initiatives that enhance climate resilience and promote sustainable practices (Fallasch and Siemons 2020). By tapping into these resources, Fiji's sugarcane industry can secure the financial support needed to integrate modern agricultural technologies effectively.

1.8 Policy Considerations and Recommendations for the Fiji Sugar Industry

The Fiji sugar industry, a cornerstone of the nation's economy and a vital source of livelihood for rural communities, is currently facing complex challenges stemming from pathogen pollution, climate change, and the apparent need for advanced yet sustainable agricultural technologies. Addressing these interconnected systemic issues is critical for safeguarding the future of the industry. This section outlines high-level policy considerations and enhanced actionable recommendations for industry experts, policymakers, and researchers dedicated to improving Fiji's sustainable sugarcane breeding and production practices.

1.8.1 Understanding the Challenges

The sugar industry in Fiji is grappling with several formidable challenges. Pathogen pollution has become a significant threat, as diseases like sugarcane smut and leaf scald can severely diminish yields. These diseases are exacerbated by global trade and climate variability, necessitating a proactive approach to managing pathogen risks effectively. Compounding the issue is climate change, which is increasingly impacting Fiji through rising temperatures, altered rainfall patterns, and extreme weather events. These changes threaten sugarcane yields, disrupt harvesting cycles, and make crops susceptible to pests and diseases. Given that Fiji's economy relies heavily on sugar production, these threats underscore the urgency for strategic policy interventions. Additionally, while advancements in agricultural technology, such as genetic modification and precision agriculture, offer promising solutions for improving productivity and resilience, they also present regulatory and social acceptance challenges. Policymakers must balance embracing innovation and addressing public concerns to ensure that these technologies can be effectively integrated into existing practices.

To address these pressing challenges and secure the future of the Fiji sugar industry, several recommendations are proposed:

1.8.2 Strengthening Research and Development (R&D)

Continuous investing in R&D is fundamental to overcoming challenges in the sugar industry. A dedicated focus on breeding programs prioritizing disease resistance and climate adaptability is essential, as weather parameters and conditions change over time. Collaborative research initiatives among local universities, agricultural institutes, and international partners should consider weather and climate deviations that can accelerate the development of resilient sugarcane varieties. Additionally, integrating traditional knowledge and practices can enhance R&D efforts, fostering a holistic approach to breeding that resonates with local farmers.

1.8.3 Enhancing Biosecurity Measures

Establishing robust and heightened biosecurity protocols is crucial for preventing the introduction and spread of pathogens. Comprehensive surveillance systems to monitor pest and disease outbreaks and stringent quarantine measures for imported plant materials should be developed. Engaging farmers in biosecurity training programs can empower them to recognize and report disease symptoms promptly, facilitating early intervention and mitigating potential losses.

1.8.4 Promoting Climate-Resilient Practices

Policies must prioritize the quick adoption of climate-smart agricultural practices. Encouraging farmers to implement integrated soil and water management strategies can amplify resilience to climate variability. Solid investments in infrastructure that supports efficient irrigation systems and drainage can help mitigate the adverse effects of droughts and flooding, as could the adoption of early warning systems. Moreover, promoting agroforestry and intercropping can improve soil health and biodiversity, reduce reliance on chemical inputs, and enhance farm productivity.

1.8.5 Leveraging Technology for Precision Agriculture

Adopting precision agriculture technologies can significantly enhance productivity while minimizing environmental impacts. Policies should incorporate providing access to tools such as satellite imagery, drone technology, and soil sensors, enabling farmers to make data-driven decisions that optimize resource use. Training programs focused on effectively using these technologies can maximize their benefits, improve farm management practices, and lead to higher yields.

1.8.6 Establishing a Regulatory Framework for Biotechnology

A clear and transparent regulatory framework for biotechnology, including genetically modified organisms (GMOs), is essential to foster innovation while addressing public concerns. Engaging stakeholders—including farmers, consumers, and scientists—in discussions about biotechnology can help demystify the technology and build public engagement and trust. The government should annually review and establish guidelines that promote research on genetically modified sugarcane varieties that exhibit enhanced disease resistance and climate resilience.

1.8.7 Fostering Public–Private Partnerships

Collaboration between the public and private sectors is crucial for advancing the sugar industry. Establishing partnerships that leverage resources, expertise, and technology can facilitate developing and disseminating innovative practices. Such collaborations can also enhance farmers' financing access, enabling them to invest in modern agricultural techniques and infrastructure essential for improving productivity.

1.8.8 Supporting Farmer Education and Capacity Building

To ensure the successful implementation of new policies and technologies, continuous education and capacity building for farmers are paramount. Initiatives that provide training on sustainable practices, pest management, and financial literacy can further empower farmers to adapt to changing conditions effectively. Engaging farmer cooperatives can enhance collective knowledge sharing and resource pooling, strengthening the resilience of rural communities.

1.8.9 Encouraging Sustainable Certification Schemes

Implementing new yet creative certification schemes for sustainable sugarcane production can enhance market access and consumer confidence. Policies should incentivize farmers to adopt sustainable practices by providing financial benefits for certified operations. Collaborating with international markets and prioritizing sustainability can open new avenues for Fijian sugar exports and increase the value of local products.

1.8.10 Monitoring and Evaluating Policy Impact

Establishing a sturdy framework for monitoring and evaluating the impact of implemented policies is crucial for ongoing improvement. Continuous assessment allows for timely adjustments, ensuring strategies remain relevant and effective in addressing emerging challenges. Engaging stakeholders in the evaluation process fosters transparency and encourages collective ownership of outcomes, strengthening the commitment to sustainable practices.

1.9 Conclusions and Future Prospective

Fiji's sugarcane industry is at a critical juncture, facing significant challenges from pathogen pollution and climate change, and therefore needs sustainable technological modernization. Such challenges present an opportunity to innovate and strengthen the sector. By adopting advanced biotechnological methods, such as marker-assisted selection (MAS) and genetic modification, the industry can develop disease-resistant sugarcane varieties, ensuring stable yields and reducing reliance on chemical inputs. Addressing climate change requires breeding resilient varieties and implementing integrated pest and disease management to sustain production amid environmental unpredictability.

As noted in this chapter, agrotechnological innovations, including precision agriculture, automation, and IoT applications, can revolutionize the sugarcane sector in Fiji, enhancing its efficiency and sustainability. Realizing these advancements, however, demands a unified effort from policymakers and industry stakeholders, specifically growers and international partners, fostering an environment conducive to innovation and capacity building. Fiji's ability to navigate these challenges through strategic policy interventions, technological adoption, and community engagement may help secure the future of its sugarcane industry. Embracing sustainable practices and focusing on resilience will ensure the sector's survival and position it as a competitive player on the global stage, contributing to the nation's economy and agricultural heritage.

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Chapter 2
Empowering Sugarcane Breeding with New Technologies

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Abstract In recent years, the global demand for sugar has surged due to rapid population growth, urbanization, and shifting dietary preferences, elevating the importance of sugarcane as both sugar and energy crop. With the trend for sugarcane production becoming increasingly focused on producing bioethanol and electricity to meet the growing global energy needs, there is an increased focus on activities in pre-breeding and the exploitation of accurate seed propagation that could transform sugarcane breeding. Genetically modified (GM) sugarcane has also emerged, particularly in Brazil, where *Agrobacterium*-mediated transformation (AMT) and biolistic systems have facilitated the development of transgenic plants. Diagnostic markers are now becoming indispensable for introgression programs, prerequisites for genetic diversity studies, cultivar identification, and genetic mapping. Technologies like quantitative trait loci (QTL) mapping, alongside omics approaches including genomics, transcriptomics, and proteomics, have contributed significantly to sustainable sugarcane production in response to climate change. While transcriptomic and metabolomic studies extended knowledge of gene regulation, micro-transcriptome studies carry further detailed insights into gene regulation, notably through the analysis of miRNA expression patterns that influence the posttranscriptional regulation of gene expression. Molecular breeding techniques such as marker-assisted selection (MAS) and genomic selection (GS) have advanced. Integration of biotechnology into breeding platforms may continue genetic progress,

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as supported by recent genomic developments. This chapter comprehensively explores sugarcane breeding and emerging technologies, focusing on integrating modern tools such as genomic selection, micropropagation, and genotyping by sequencing (GBS) to advance sugarcane breeding. It delves into the historical context of sugarcane cultivation and breeding, highlights the evolution of genetically modified (GM) sugarcane, and examines the critical role of omics technologies in understanding gene regulation and improving the agronomically important traits.

Keywords Micropropagation · Remote sensing · Transcriptomics · Genomic selection · Micro-transcriptome · Genotyping by sequencing · Pre-breeding

2.1 Introduction

Sugarcane is a vital crop for the biofuel industry and global sugar production, supplying around 80% of the world's sugar. In 2021, Brazil and India collectively contributed 61% of global sugarcane production. As the fifth-largest crop globally by production value and acreage, sugarcane is also the second-largest bioenergy crop (FAO 2023; Verma et al. 2024a). The domestication of sugarcane can be traced back to approximately 10,000 years, with early sweet varieties (*Saccharum officinarum*) evolving from *Saccharum robustum* (Daniels and Roach 1987; Verma et al. 2024b). Modern sugarcane, a vegetatively propagated crop, is a complex polyploid and aneuploid hybrid resulting from interspecific crosses within the *Saccharum* genus. Its origin is accredited mainly to two wild species: *Saccharum officinarum* ($2n = 80$; $x = 10$) and *Saccharum spontaneum* ($2n = 40–130$; $x = 8$). The majority of the sugarcane genome (80%) originates from *S. officinarum*, with *S. spontaneum* contributing around 10–15% and the remaining 5–10% consisting of recombinant chromosomes (Zhang et al. 2018a).

C_4 grasses, including maize, evolved from a common ancestor with other C_4 grasses like sorghum about 15–20 million years ago, and the divergence happened around 6–9 million years ago (Paterson et al. 2004, 2009; Verma et al. 2020a, b). The sugarcane genome is one of the largest of any crop, approximately 10 Gb, and it has chromosome numbers ranging from 100 to 130, with considerable variation between species. This genetic complexity poses significant challenges and is one of the reasons why sugarcane breeding is slow and intricate. Molecular tracing has revealed that the crop originated in Southeast Asia and New Guinea (Lebot 1999; Huang et al. 2024). As an exponentially growing population has fueled the global demand for sugar and bioenergy, the exploitation of sugarcane's genetic resources has reached its limits. Breeding in sugarcane is fundamentally interspecific hybridization and backcrossing among different species, which has created cultivars with highly complex genomes. Traditional sugarcane breeding relies on clonal selection, manual cane stalk weight, and sugar content assessment (Afghan et al. 2024; Xu

et al. 2024). This process typically spans 10–12 years, with disease resistance screening integrated toward the later stages. Despite these efforts, the rate of sugarcane yield enhancement has lagged behind that of other prominent crops, and some breeding efforts display stagnation in genetic gain (Singh et al. 2019; FAO 2023).

Several factors have hindered this progress to date. These include long breeding cycles, complexity of large-scale phenotyping, low narrow-sense heritability for many key traits, and challenges related to the large and complex polyploid genome of the crop. Moreover, genotype–environment interactions further complicate the breeding process (Xiu-Peng Song et al. 2019; Verma et al. 2021a, 2024c, d; Deng et al. 2023; Chen et al. 2023a, b). The significant biomass of sugarcane complicates accurate phenotyping, making selection less precise and raising concerns about the effectiveness of conventional phenotyping methods (Verma et al. 2020b, c; Som-Ard et al. 2021; Bhatt et al. 2024).

Cost-effective and high-throughput advanced phenotyping techniques are essential for precisely determining the true potential for yield in sugarcane clones in breeding programs. These advancements could significantly improve selection accuracy and fast-track the breeding process. Furthermore, with the help of DNA marker systems, modern breeding technologies have evolved over time (Fig. 2.1). Earlier hybridization-based markers have now been replaced by single nucleotide polymorphism (SNP) markers, made possible by cutting-edge next-generation sequencing (NGS) technologies. Gene introgression through intergeneric hybridization has also been crucial in sugarcane breeding. Successful hybrids have been produced with species like *Erianthus* (Ram et al. 2001), *Miscanthus* (Kar et al. 2019), *Narenga* (Chang et al. 2020), and *Sorghum* (Hodnett et al. 2010). Due to

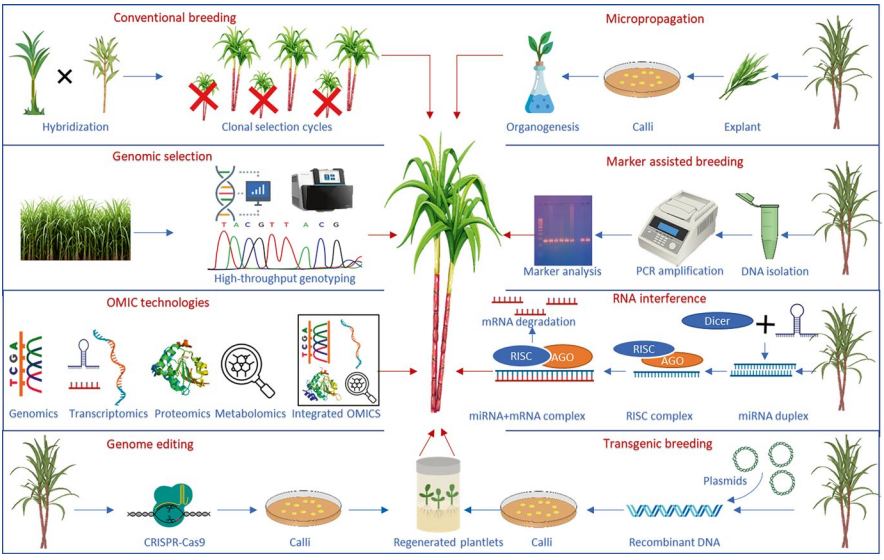

Fig. 2.1 Advanced approaches in sugarcane breeding and genetic enhancement

their vigor, fertility, and ability to be propagated through vegetative means, these hybrids are classified as perennial. Over the past decade, genetic transformation techniques and various omics technologies have drawn much attention and provided new avenues for research pathways in sugarcane improvement (Verma et al. 2023a, b; Liang et al. 2024). Transgenic protocols, including *Agrobacterium*-mediated transformation (AMT) and the biolistic method (Ramasamy et al. 2018), have been utilized for genetic modifications in sugarcane.

The greatest breakthroughs have been made in recent years, with modifying sugarcane genomes involving adding, deleting, or replacing specific gene sequences with genome editing (GE) technologies, especially the clustered regularly interspaced short palindromic repeats/CRISPR-associated protein 9 (CRISPR/Cas9) system (Mohan et al. 2022; Malviya et al. 2024). Rapid advances in genome sequencing and marker technologies have accelerated the establishment of genotyping systems such as genomic selection (GS), which has improved marker–trait association (MTA) studies (Meuwissen and Goddard 2001; Li et al. 2024). Genomic selection utilizes a large number of trait-associated DNA markers, such as SNP markers, distributed throughout the entire genome, providing robust predictions for selecting elite sugarcane clones resistant to both biotic and abiotic stresses (Verma et al. 2020d, 2020e, f; O'Connell et al. 2022; Chen et al. 2023a, b; Jagadesh et al. 2024). However, the full potential of GS in sugarcane breeding remains to be realized; prediction models still need refinement to accommodate changes in genetic backgrounds and diverse agroecological conditions.

Omics technologies, broadly encompassing genomics, transcriptomics, proteomics, and metabolomics, continue to reveal the complex interactions between metabolites, proteins, and genes. These tools are instrumental in unraveling the molecular mechanisms that control yield and stress resistance in sugarcane (Verma et al. 2019a, b; Viswanathan et al. 2022). With an emphasis on micropropagation, genome editing, RNA interference, transgenics, genotyping by sequencing (GBS), genomic selection (GS), and multi-omics techniques for sugarcane improvement, this chapter gives a summary of the new technologies enabling sugarcane breeding. These modern breeding techniques are critical for the rapid improvement of sugarcane in response to the challenges of contemporary agriculture.

2.2 Breeding Techniques and Trait Improvement

Traditional breeding methods for sugarcane improvement include introduction, clonal selection, backcrossing, interspecific and intergeneric hybridization, and advanced techniques like tissue culture (micropropagation) and mutation breeding. Distant hybridization transfers biotic and abiotic stress-resistant genes from wild species to cultivated varieties, while mutation breeding focuses on developing disease-resistant strains. The genus *Saccharum* includes the species *S. officinarum*, *S. robustum*, *S. spontaneum*, *Saccharum barberi*, *Saccharum sinense*, and *Saccharum edule* as well as allied species from the genera *Erianthus*, *Miscanthus*,

Narenga, and *Sclerostachya*, collectively known as the "*Saccharum* complex." Northeast India and the Himalayan region are biodiversity hotspots for *S. spontaneum* and related genera like *Miscanthus*, *Erianthus*, *Narenga*, and *Sclerostachya* (Nair et al. 1991; Govindaraj et al. 2010; Singh et al. 2020; Guo et al. 2021).

Genetic variation in sugarcane breeding comes from various species, and the process typically involves three stages: selecting parents, pollination, and progeny selection. Parent selection is critical, employing both biparental and polycross methods, with biparental crosses generally yielding more consistent outcomes. Offspring are selected over several years, during which thousands of genotypes are evaluated for traits such as sugar content and disease resistance, with the focus gradually narrowing down to yield and adaptability (Chen et al. 2020; Singh et al. 2021a, b; Verma et al. 2021b, 2024e). In breeding programs, selection can be individual-based, where traits with high heritability, like disease resistance, are prioritized, or bulk-based, where the average phenotype of entire progenies is considered (Anas et al. 2021a, b). Sequential selection further enhances genetic gain by selecting individuals from the top-performing families. Moreover, regional selection ensures that traits like drought tolerance and ratooning capacity are optimized to suit local environmental conditions (Meena et al. 2020; Malviya et al. 2020; Verma et al. 2023c, d)

The primary objectives of conventional sugarcane breeding programs focus on enhancing productivity, adaptability, and sustainability. One of the key goals is to increase sugar and cane production by maximizing sugar and biomass yield per unit area. This involves improving stalk characteristics like height, diameter, and number, as these traits directly impact sugar content and biomass, particularly for bioenergy uses like second-generation biofuels (de Morais et al. 2015; Malviya et al. 2021; Huang et al. 2022). Another important objective is improving ratoon performance, which enables sugarcane to regrow after harvesting, ensuring continued high productivity in successive ratoon cycles and thereby boosting economic efficiency (Verma et al. 2021c, 2022a, b). Developing stress-tolerant varieties is another priority, particularly in addressing abiotic stresses, which include drought, salinity, and nutrient-poor soils (Verma et al. 2022c, d). These improvements enhance sugarcane cultivation in marginal areas, reduce irrigation needs, and optimize productivity under challenging environmental conditions (Sharma et al. 2021; Krishna et al. 2022).

Disease resistance is also a significant focus, with breeding programs targeting diseases like smut, rust, and ratoon stunting as well as viral pathogens, including the sugarcane mosaic virus (SCMV) and sugarcane yellow leaf virus (SCYLV). Another significant goal is to improve bioenergy production by breeding varieties with higher fiber content and improved cell wall digestibility, making sugarcane more efficient for bioethanol and other bioenergy applications (Singh et al. 2021; Oliveira et al. 2022; Guo et al. 2023).

Pest tolerance is equally important, with efforts to reduce yield losses from pests like the root froghopper, sugarcane weevil, and sugarcane borer, promoting more sustainable and eco-friendly farming practices (Verma et al. 2021d, e, 2022e). In terms of developmental and physiological traits, breeding programs aim to enhance

traits such as stalk height, diameter, tillering, and leaf angle, which are essential for both biomass production and mechanical harvesting (Verma et al. 2021f; Liang et al. 2024). Controlling flowering induction also helps maintain sugar accumulation by ensuring energy is used for growth rather than reproduction. Nitrogen use efficiency (NUE) and mycorrhizal associations are additional traits of interest, reducing reliance on fertilizers and lowering production costs while enhancing environmental sustainability (Juntahum et al. 2020; Verma et al. 2022f, g). To overcome limitations in traditional breeding, molecular techniques have been integrated into sugarcane breeding. Marker-associated selection (MAS) and genomic selection (GS) have been employed to identify and select desirable traits, with the latter showing great potential for accelerating genetic gain by shortening breeding cycles and improving clonal performance predictions. Genetic engineering approaches, such as *Agrobacterium*-mediated transformation, are also being explored to introduce economically important traits into sugarcane (Wu et al. 2022; Xu et al. 2022; Li et al. 2023a, b).

2.2.1 Micropropagation in Sugarcane

Traditional breeding methods for developing new varieties are time-consuming, typically taking 8–10 years, followed by another 10–12 years for distribution in remote areas, and, by that time, the yield and quality of the varieties may start to decline. However, sugarcane can be clonally propagated, allowing modern varieties to maintain their highly heterozygous hybrid nature. Still, conventionally, it will require multiple generations to produce sufficient material, hindering the use of advanced genomic techniques. Clonal propagation is multiplying genetically identical plants, known as clones. When this is done using tissue culture techniques, it is commonly referred to as micropropagation. These microplants can be generated via shoots or meristems, callus, or explants from the mother plant or somatic embryogenesis. Micropropagation allows the mass production of thousands of plants in a controlled laboratory environment (Sajid and Pervaiz 2008; Guo et al. 2022).

This technique offers several advantages, including the rapid multiplication of elite lines for large-scale field trials and the quick production of clonal material for growers of newly released varieties within just 1–2 years, complementing conventional seed production (Ming et al. 2006; Joshi et al. 2022). Bioreactors are also effective in mass seed production via micropropagation (Murthy et al. 2023). These closed, sterile systems use liquid nutrient media and optimized environmental controls (Leathers et al. 1995). Temporary immersion system (TIS) bioreactors quickly multiply seed material and facilitate transgenic propagation for faster phenotypic evaluations (Ramasamy et al. 2018; Singh et al. 2022). It reduces time, labor, and costs while enhancing plant growth and nutrient absorption. They yield high-quality plantlets, achieving up to 1,000 plants compared to traditional methods (da Silva et al. 2020). In vitro studies in sugarcane began in the late 1970s, leading to the

development and standardization of protocols for micropropagation (Sreenivasan and Jalaja 1979; Baday 2020; Salokhe 2021; Di Pauli et al. 2021; Tayade et al. 2021).

Additionally, since plants are propagated from small, clean explants such as shoot tips or meristems, they are disease- and pest-free, minimizing associated losses. Somaclones produced through tissue culture have led to increased productivity and elimination of defects such as spines on leaf sheaths (Co 7717), leaf drying (Co 7704), and disease susceptibility, including the eradication of SCMV through heat therapy along with meristem culture. The Sugarcane Breeding Institute (SBI), Coimbatore, has developed coenzyme A (CoA) 7601 and CoC 671 via tissue culture to eliminate rust and smut, respectively. It is also beneficial for rejuvenating old and degenerated varieties, potentially eliminating endogenous pathogens, with these advantages already demonstrated in farmers' fields (Malviya et al. 2022; Bhatt et al. 2023a, b). Tissue culture enables the assessment of plant stress tolerance under controlled in vitro conditions, bypassing whole-plant regulatory mechanisms (Lutts et al. 2004).

Studies on sugarcane have revealed that stress-resistant genotypes exhibit lower proline content and better regeneration, making sugarcane an ideal candidate for breeding drought-resistant varieties with early maturity and high sugar yield (Singh 2003; Karpe et al. 2012; Abdelsalam et al. 2021; Kumari et al. 2022). Some examples of somaclonal varieties that exhibit high sugar recovery are Co 92007, Co 92029, Co 94003, Co 99011, Co 99012 and Co 7704 (resistant against smut and one strain of red rot), Co 88026, Co 93005, Co 94012 and Co 95016 (Nair 2011; Tiwari et al. 2015). VSI 434, developed through callus culture, is drought-tolerant and has a high sugar recovery rate (Tawar et al. 2016). Despite its advantages, micropropagation in sugarcane faces challenges that limit widespread adoption. Tissue-cultured crops often produce thinner canes, with high production costs and low regeneration rates further hindering its popularity. However, using small explant sections, the thin cell layer (TCL) technique has shown promise in improving differentiation and regeneration (Iqbal et al. 2023). Microbial contamination, especially from *Enterobacter* and *Bacillus* spp., can lead to significant culture loss (Dukoon et al. 1995), while phenolic oxidation during culture initiation impedes growth and causes abnormal cell development (North et al. 2012; Song et al. 2022). Vitrification, which causes thick stems and glassy leaves, complicates rooting and establishment (Constantine 1986). Remedies, such as open, sugar-free tissue culture systems, have been proposed (Lu et al. 2020).

Additionally, somaclonal variation, chromosomal mosaicism, and chimerism contribute to genetic diversity, complicating true cloning (Heinz et al. 1969; Sreenivasan and Jalaja 1995). Molecular tools like DNA markers can help manage this variation. While biotechnological breeding faces challenges in plant regeneration, certain sugarcane genotypes can be regenerated via indirect somatic embryogenesis, making them suitable for breeding (Di Pauli et al. 2021). A three-tier seed multiplication program can help maintain genetic uniformity and ensure the production of certified seed cane, which would support the broader use of tissue culture and lower costs (Lal et al. 2015; Sharma et al. 2022). Conducting field trials to

compare micropropagated plants with those grown conventionally is crucial for evaluating traits that influence sugar yield.

2.2.2 Molecular Markers for Sugarcane Breeding

Molecular markers are DNA fragments that are associated with a particular trait. For the genetic analysis of sugarcane, several molecular markers have been extensively used, such as RAPD (random amplified polymorphic DNA), AFLPs (amplified fragment length polymorphisms), RFLPs (restriction fragment length polymorphisms), KASP (kompetitive allele-specific PCR), SNPs (single nucleotide polymorphisms), ISSRs (inter simple sequence repeats), TRAPs (target region amplification polymorphisms) and SSRs (simple sequence repeats), and DArTs (diversity arrays technology) (Palhares et al. 2012; Ali et al. 2019a; Manimekalai et al. 2020; Singh et al. 2023). The initial genetic maps for sugarcane were developed using RFLP markers, but these maps were sparse and unevenly distributed across the genome (Silva et al. 1993; Ming et al. 1998, 2002). In the second phase of marker development, PCR-based markers made it easier to generate a large number of markers, although these were manually scored as the dominant markers. RAPD markers, while fast to produce, had poor reproducibility and limited transferability across populations and laboratories. However, RAPD markers helped detect genetic variability within cultivars (Nair et al. 2002) and between *Saccharum* species (Nair et al. 1999), and they also contributed to RFLP genetic linkage maps by increasing marker density and genome coverage (da Silva et al. 1995).

AFLP markers, another PCR-based system, were more reproducible than RAPD markers, as they were dispersed randomly across the genome. Later, the TRAP technique, an adaptation of AFLP, leveraged advances in sequencing technologies (Alwala et al. 2008). Among the first molecular markers developed for sugarcane were simple sequence repeats (SSRs) or microsatellites (Cordeiro et al. 2003). Like RFLPs, SSRs revealed multiple homozygous alleles with a single primer set, but their lower complexity allowed for automated scoring through capillary sequencing platforms, improving genotyping accuracy. SSRs were later integrated into AFLP genetic maps, providing essential information on linkage groups within homologous groups. Additionally, DArT markers were introduced as the first array-based marker system for sugarcane. Sugarcane breeding programs rely heavily on PCR-based markers, especially SSRs, for clonal identity testing during the propagation stage (Piperidis and Taylor 2001; Piperidis et al. 2004; Joshi et al. 2023). Microsatellite DNA markers were also used to assess the genetic diversity of sugarcane germplasm, using phenotypic traits and pedigree data (Cordeiro et al. 2003).

AFLP, SSRs, and TRAPs are DNA markers that successfully identify molecular-level genetic variations independent of environmental influences (Creste et al. 2010). Recent marker development in sugarcane has capitalized on high-throughput sequencing technologies initially developed for diploid crops. These technologies exploit abundant polymorphic SNPs and insertions/deletions distributed throughout

plant genomes. Early SNP markers were derived from expressed sequence tag (EST) collections, with genotyping conducted via Sequenom assays. Two major SNP genotyping platforms have since emerged: array-based SNP genotyping, which necessitates extensive sequence information, and GBS, which does not need prior sequence information but depends heavily on bioinformatics tools. Molecular markers have been the cornerstone of advancing sugarcane breeding programs, facilitating the improvement of multiple traits in sugarcane (Table 2.1). Sugarcane breeding mainly relies on phenotypic selection, involving mass and family selection, with long crop cycles and extensive evaluations. Faster genetic gain is hindered by a narrow genetic base, complex traits, and extended breeding cycles (Raboin et al. 2008; Wei and Jackson 2016). Although the intricacy of the sugarcane genome has hindered the application of molecular markers (Grivet and Arruda 2002; Costet et al. 2012), genomic selection presents a viable substitute to hasten genetic advancement for quantitative traits.

2.2.3 Array-Based SNP Genotyping

This technique enables the genotyping of several tens of thousands of SNP markers with data processing requiring only basic computer skills. It is appropriate for both diploid and polyploid crops, including sugarcane. The arrays are fixed once they are created and developed, and they need sequence data specific to the germplasm they are intended to be used on. In sugarcane, the development of arrays is further complicated by the necessity to identify low-dose SNPs since many high-dose SNPs are not polymorphic across breeding lines (Aitken et al. 2016). Aitken et al. (2016) developed the first fixed array for sugarcane. They identified 4 million SNPs by sequencing 15 key ancestors and parent clones, filtering out high-dose markers in the process. This array of more than 345,000 SNPs was used to screen 480 breeding lines for markers responsible for cane yield and commercial cane sugar. Garsmeur et al. (2018) created a second, smaller Affymetrix Axiom array containing 58,000 SNP variants, primarily low-dose markers. Several studies have used this method for genetic mapping and genomic selection (Yadav et al. 2020; Deomano et al. 2020).

2.2.3.1 Genotyping by Sequencing

Since its advent in the early twenty-first century, next-generation sequencing (NGS) has been extensively employed for whole genome sequencing, resequencing, quantitative trait loci (QTL) mapping, and genome-wide association studies (GWASs) (Smith et al. 2008; Varshney et al. 2009; Li et al. 2009). However, its application to species with massive, complex genomes, like sugarcane that has an estimated genome size of 100 Gb, presents unique challenges. Analyzing sugarcane's genome size, which can vary between 3.80 and 10.96 Gb across different varieties with a ploidy of approximately $12x$ and $2n \approx 114$ chromosomes, is challenging due to the

Table 2.1 Molecular markers and strategies applied in sugarcane breeding, demonstrating their relevance in trait improvement and genetic diversity studies

Molecular markers	Marker count	Target trait	Key findings/observations	References
SNP	183,842	Sugarcane orange rust (SOR)	Identified six QTLs (Oru1–Oru6) linked to SOR resistance, with most originating from *S. spontaneum*	Dijoux et al. (2024)
EST-SSR	30	Sugar yield	SEM 407 marker associated with Brix% and sucrose content was identified, linked to diphosphate—fructose-6-phosphate 1-phosphotransferase, suggesting its potential use in MAS	Divakar et al. (2023)
SNP	7	Sugar and yield components	Validated SNP markers mSoSUS1_SNPCh10.T/C and mSoKAN1_SNPCh7.T/C significantly associated with traits like pol, brix, CCS (Commercial Cane Sugar), fiber, and yield were identified	Khanbo et al. (2023)
SSR and AFLP	91	Sugarcane mosaic virus (SCMV)	Among 98 sugarcane genotypes, 24 significant markers were identified for SMD resistance, explaining up to 29.95% of trait variation	Burbano et al. (2022)
SNP and KASP	148,500	Smut (*Sporisorium scitamineum*)	Mapped 21 major QTLs for smut resistance and converted 77 SNPs to KASP markers, with 5 significantly linked to smut resistance	Gao et al. (2022)
SNP	82	Ratoon stunting (*Leifsonia xyli* subsp. xyli)	Identified 23 QTLs linked to disease resistance, explaining 6–13% of phenotypic variation	You et al. (2021)
TRAP	823	Sugar and lignin metabolism	High polymorphism information content found in wild relatives; modern cultivars showed inherited variability for sugar metabolism genes from *S. officinarum* and *S. spontaneum*	Junior et al. (2020)
EST-SSR, AFLP, and SSR	775	Sugar-related traits	Six QTL regions were identified through multiple QTL mapping; wo EST-SSR markers were validated and associated with UDP glycosyltransferase and beta-amylase	Ukoskit et al. (2019)
SSR, eSSR, and SNP	1948	Leaf scald (*Xanthomonas albilineans*)	Identified eight QTLs associated with resistance; comparative analysis with sorghum pinpointed three SNPs contributing to 16% variance	Gutierrez et al. (2018)
SNP	2453	Sugarcane yellow leaf virus (SCYLV)	Two QTLs were identified for SCYLV resistance; major QTL qSCYLVR79 linked to marker 3PAV3154 accounted for 23% variation	Islam et al. (2018)

(continued)

Table 2.1 (continued)

Molecular markers	Marker count	Target trait	Key findings/observations	References
AFLP	55	Drought tolerance	Revealed 55 polymorphic markers out of 886 total amplicons; also highlighted tolerant and sensitive cultivars	Khaled et al. (2018)
AFLP, SSR, and EST-SSR	488	Brown rust (*Puccinia melanocephala*)	Identified 61 quantitative trait alleles (QTA) for brown rust resistance across plant cane and ratoon stages, with 10 shared between both	Santos et al. (2015)
AFLP and SSR	1405	Spotted stem borer (*Chilo sacchariphagus*)	Quantitative trait allele (QTA) mapping revealed nine QTAs explaining 42% of phenotypic variance, with two QTAs co-localizing with resistance gene analog clusters	Nibouche et al. (2012)

SMD sugarcane mosaic disease.

requirement of high sequence coverage (D'Hont 2005; Bao et al. 2024; Healey et al. 2024). To address this complexity, techniques like target enrichment and restriction enzyme digestion have been developed to reduce genome size for sequencing. Target enrichment, using methods such as PCR amplification and hybrid capture, isolates specific genomic regions based on reference sequences but relies heavily on existing genomic data (Yang et al. 2017a).

Genotyping by sequencing (GBS) is a cost-effective, multiplex sequencing technique that creates reduced representation libraries specifically designed for the Illumina next-generation sequencing (NGS) platform, utilizing a barcoding system and thereby enhancing NGS efficiency (Elshire et al. 2011). In GBS, the genomic DNA undergoes digestion using a restriction enzyme, typically a frequent cutter. A common adapter and a barcoded adapter are both ligated to the resulting fragments, allowing for efficient fragment amplification (Fig. 2.2). After pooling the fragments, they are bridge amplified on an Illumina flow cell, and amplification takes place of only those fragments that have different adapters on both their ends. For the purpose of SNP calling, one end of each fragment is sequenced, and the reads are mapped to a reference genome. It is not indispensable to have a complete reference genome. Instead, the consensus from the reads can be used as a reference. To target specific genomic regions and reduce complexity, methylation-sensitive enzymes can be employed to avoid repetitive sequences and enrich low-copy regions (Fig. 2.2).

Two restriction enzymes: a rare cutter (e.g., PstI) and a common cutter (e.g., MspI) further reduces complexity, and the barcoded adapter is then affixed to the rare-cutter fragment (Poland et al. 2012). GBS allows the sequencing of several samples with only 100 ng DNA as input. It is invaluable to genetic mapping and breeding applications, as it allows for the imputation of missing SNPs within haplotype blocks, thus minimizing costs (He et al. 2014). It offers an integrated marker discovery and genotyping process without requiring a reference genome (Deschamps

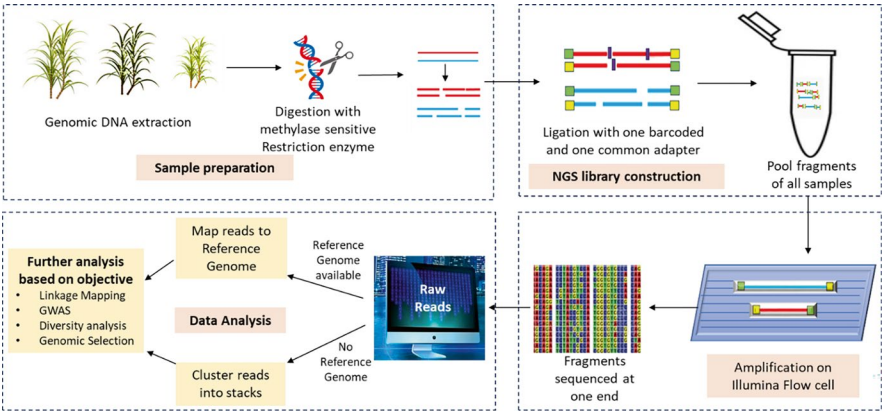

Fig. 2.2 Workflow of the genotyping by sequencing approach in sugarcane (purple box: restriction sites (*Pst*I, *Msp*I, *Ape*KI); green box: a barcoded adapter; yellow box: a common adapter; amplification of only those fragments that have ligated both the barcoded adapter and common adapter)

et al. 2012), making it particularly effective for non-model species like sugarcane (Yang et al. 2017b). Traditional genetic markers are often specific to certain populations, causing allelic bias, especially in genetically diverse groups like wild sugarcane germplasm (Deschamps et al. 2012; Zheng et al. 2023), but eliminate ascertainment bias by directly using data from the populations being genotyped (Elshire et al. 2011). The advancements in NGS technology and reduced costs have aided in resequencing whole populations instead of just a limited individual, simultaneously enabling genome-wide identification and scoring of tons of markers.

The repeatome in plants (Flavell et al. 1994) can pose significant difficulties in identifying variants in de novo assembly, reference sequence alignment, and sequence comparison. The decision to sequence the whole genome or a reduced portion is typically influenced by factors such as repetitive content, the presence or absence of homeologs, and ploidy level (SanMiguel et al. 1996; Bennetzen et al. 2005). Polyploid species possess homoeologous genomes, resulting in multiple similar gene copies. This leads to detecting SNPs (hemi-SNPs) caused by differences in paralogous gene copies, creating artificial polymorphisms. Strategies like reduced representation resequencing and identifying SNPs in diploid progenitors have been applied in polyploid species like maize (Elshire et al. 2011), wheat (Poland et al. 2012; Trick et al. 2012), and sugarcane (Singh and Singh 2015; Healey et al. 2024). When integrated with phenotypic data, GBS methodologies furnish a robust foundation for the expedited mapping and gene identification for desirable characteristics that can subsequently be incorporated into species germplasm.

Balsalobre et al. (2017) were among the first to utilize GBS for extensive variant discovery in sugarcane, generating many markers that facilitated ploidy and allelic dosage estimations, besides identifying QTLs for molecular breeding. This method has helped identify 84,000 SNPs across different sugarcane accessions (Yang et al.

2017a; Balsalobre et al. 2017). The sequence-based molecular markers produced through GBS enable the construction of genetic maps without requiring a reference genome (He et al. 2014). High-density genetic maps derived from GBS data allow researchers to identify loci of interest across various populations, including segregating populations and mutant pools. More than 4,600 SNP markers from GBS have been used in sugarcane to create high-density genetic maps (Yang et al. 2017b). Additionally, GBS-derived markers have been critical in establishing marker–trait associations for key characters like sucrose content and cane yield (Fickett et al. 2019). The method has also been effective in discovering SNPs in diverse genomic contexts, including methyl-filtered genomes and RNA sequencing (RNA-Seq) transcriptomes (Cardoso-Silva et al. 2014).

A specialized bioinformatics pipeline is required to address the challenges of analyzing large datasets from complex polyploid species without a reference genome, which can create computational constraints, while identifying allelic variations considering both homo- and homeologous chromosomes. It is important to note that the analysis may exhibit discrepancies across populations, resulting in significant missing data. Due to the large genome and high ploidy, it becomes imperative to increase read depth for precise variant detection (Elshire et al. 2011; Solanki et al. 2023). Furthermore, restriction enzyme-based GBS methods often yield inconsistent SNP information across samples, limiting their utility for SNP discovery. To address this issue, imputation models such as BEAGLE v3.0.2 and IMPUTE v2 have been developed (Singh and Singh 2015). GBS has advantages over SNP arrays, particularly in identifying causal mutations and improving prediction accuracy when rare alleles contribute significantly to genetic variance (Manimekalai et al. 2020). In contrast, array-based sugarcane sequencing requires substantial sequencing data for adequate SNP coverage, which can then be analyzed for reliable genotyping. Determining allele dosage in allopolyploid organisms like sugarcane is challenging due to their complex genomic structure, which includes multiple sub-genomes from different species (Piperidis et al. 2020; Aitken 2022). This complexity increases the risk of genotype determination errors, particularly from structural variations between homologous alleles that may lead to preferential binding of primers (Makhoul et al. 2020).

However, combining GBS with restriction site-associated DNA sequencing (RAD-seq) allows for distinguishing between homologous alleles, providing a more precise estimation of SNP allele dosages. Converting SNP markers identified through GBS and RAD-seq into functional assays remains challenging in polyploid crops like sugarcane, where only 20–25% of the markers are effectively transferable (McNeil et al. 2017; Kumari et al. 2023). Flex-Seq has shown more excellent reliability in accurately reproducing allele dosages from GBS and RAD-seq (Manimekalai et al. 2020; Tian et al. 2023; Jaimes et al. 2024). GBS presents a promising tool for various genetic studies, including linkage map construction, physical mapping, association mapping, genetic diversity assessments, phylogenetic analyses, and MAS and GS in sugarcane. When combined with GBS, the GS procedure is employed to enhance prediction accuracies for polygenically inherited characters and improve the crop selection procedure (Faville et al. 2018).

2.2.4 Genomic Selection

Genomic selection (GS) involves techniques that utilize genetic information across the entire genome to accurately predict traits, enabling selection based solely on these predictions (Meuwissen et al. 2001). GS focuses on predicting phenotypes solely using genotypic data, allowing breeders to select individuals with the highest breeding values. The key benefit of GS is its ability to identify numerous genetic factors with minor effects while concurrently enhancing various phenotypic traits. The process involves training populations (TPs), which provide genotypic and phenotypic information to develop predictive models. Once the model is trained, it predicts genomic estimated breeding values (GEBVs) in breeding populations (BPs) where only genotypic data are available, enabling selection decisions based on genetic potential alone. The success of GS depends on the genetic similarity between the training and breeding populations, particularly in terms of linkage disequilibrium between the marker loci associated with the trait. Diversified, well-phenotyped, and genotyped lines serve as robust TPs to calibrate prediction models for accurate GEBV estimation, enabling more efficient breeding progress (Meuwissen et al. 2001; Heffner et al. 2010; Song et al. 2021).

This shift from phenotype-based selection to genotype-driven decisions increases selection intensity and shortens breeding cycles. The rise in cost-effective DNA marker detection methods, such as GBS and SNP arrays, has made GS more accessible. Still, its success in breeding programs depends on multiple factors, including the genetic makeup of characters, heritability, and financial resources. GS is still not widely implemented in sugarcane. However, studies in tetraploid potatoes (Slater et al. 2016; Endelman et al. 2018), blueberries (de Bem Oliveira et al. 2019), and octoploid strawberries have shown that their effectiveness depends on the genetic architecture of the traits (Zingaretti et al. 2019). Applying GS in sugarcane breeding presents several challenges due to the complex biology and genetics, complicating large-scale phenotyping efforts. Additionally, the genome of sugarcane is one of the most challenging among crops, which makes genotyping difficult. A key issue with GS models, originally created for seed-propagated plants, is their focus on additive effects, often treating dominance and epistatic effects as superfluous.

However, in micropropagated plants like sugarcane, these effects significantly influence trait inheritance. The entire allele set, including interactions, is passed to the next generation, making nonadditive effects crucial for accurate predictions. Another challenge arises from allele dosage, where multiple copies of an allele in sugarcane may affect trait expression, but standard SNP models only consider the presence or absence (Sandhu et al. 2022). Furthermore, genotyping costs remain high in sugarcane, limiting large-scale genomic evaluations. Alongside genotyping, there is a need for high-throughput precise phenotyping in multiple environments, as genotype–environment interactions can reduce the accuracy of prediction. Including environmental parameters could improve heritability estimates, and incorporating allele dosage and nonadditive effects into GS models would help create better prediction models. Recent advancements in SNP markers, capable of

covering the entire genome and utilizing allelic dosage data, have made GS more applicable to polyploids (Hackett et al. 2013; Garcia et al. 2013).

Gouy et al. (2013) demonstrated the potential of GS in sugarcane by genotyping 334 clones from breeding programs in Reunion Island and Guadeloupe, assessing 10 agronomic traits. Prediction accuracy rates varied between 0.11 and 0.62 within panels and between 0.13 and 0.55 across panels using four genomic models—ridge regression, Bayesian lasso, partial least squares regression, and reproducing kernel Hilbert space—highlighting the promise of GS even with small training populations. To incorporate GS into sugarcane breeding (Fig. 2.3), a key strategy involves enhancing genetic gain by overcoming the bottleneck of allele recombination, otherwise restricted to the beginning of the breeding cycle. Recurrent genomic selection (RGS) offers a promising solution by accelerating selection and crossing elite germplasm throughout the cycle, enabling quicker genetic improvement by simultaneous line development and population improvement. It can achieve 2.5 times more genetic gain than traditional methods (Voss-Fels et al. 2019).

For long-term genetic gain, reciprocal recurrent selection (RRS) improves general and specific combining abilities by optimizing crossbred populations, as shown in maize and oil palm (Comstock et al. 1949). Voss-Fels et al. (2021) performed a simulation study in sugarcane, highlighting the possibility of GS substantially boosting genetic gain for quantitative characters. A modified RRS version incorporating GS could improve sugarcane breeding by using a reference population and genomic prediction models to select the best progeny from diverse parent lines (Yadav et al. 2020). This method allows for ongoing improvement cycles through RGS, leading to better-crossbred clones. Challenges include high costs for genotyping and the need for constant updates to prediction models to account for changes in

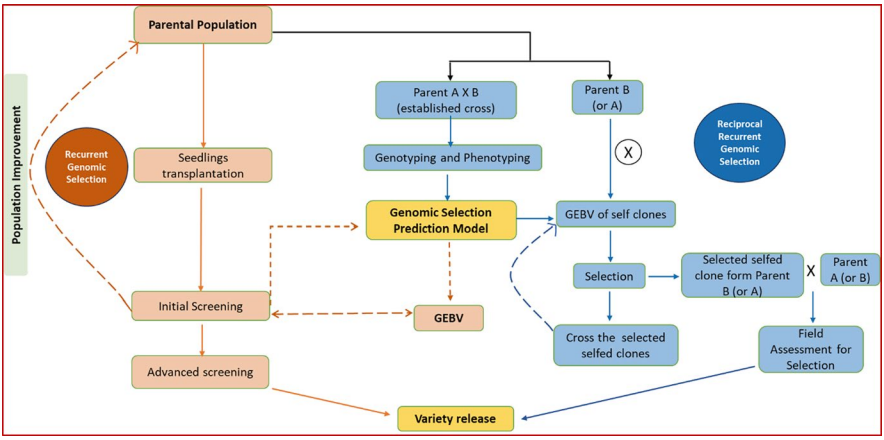

Fig. 2.3 A flowchart showing recurrent genomic selection (RGS, orange) and modified reciprocal recurrent genomic selection (RRGS, blue) in sugarcane. In RGS, a prediction model is created using data from earlier trials. In RRGS, the model is built using crosses between unrelated lines, and selfed progeny are screened based on their genomic estimated breeding values (GEBVs) before being crossed with a different parent. (Adapted from Yadav et al. (2020))

allele frequencies. Additionally, introgression of novel alleles from wild gene pools could replenish genetic diversity in cultivated sugarcane, though this is time-consuming (Ødegård et al. 2009).

Pre-breeding in sugarcane typically requires three or more backcrosses with recurring parent lines. Wild species (*S. spontaneum*, *S. barberi*, and *S. robustum)* are used as male parents, while *S. officinarum* or improved varieties act as females. The hybrids are developed from them in the first cycle, while the second cycle involves backcrossing the progeny obtained with noble cane. GS strategies can be applied in both cycles, utilizing genomic markers and specific models to identify true F1 individuals in the first cycle. These individuals are then trained during each backcross to improve the accuracy of genomic predictions (Mahadevaiah et al. 2021). Simulation studies are essential for designing cost-effective GS strategies, while empirical validation will ensure practical applicability. By combining GS with GWASs, sugarcane breeding programs can better capture both large- and small-effect QTLs, enhancing genetic gain over time (Olatoye et al. 2019). Further research is required to refine RGS and reciprocal recurrent genomic selection (RRGS) strategies for sugarcane, but they hold significant promise for long-term genetic improvement.

2.2.5 Genome Editing in Sugarcane

Sugarcane genome sequencing has accelerated crop improvement by allowing the identification of critical genes associated with valuable agronomic traits (Table 2.2) as well as the implementation of precise techniques for genome editing that include transcription activator-like effector nucleases (TALENs), clustered regularly interspaced short palindromic repeats (CRISPR), and zinc-finger nucleases (ZFNs) for sequence-specific genetic alterations. The field of molecular biology has significantly benefited from genome editing (GE), which has developed biotechnological tools for gene alteration that aim to introduce new cultivars with improved traits, such as increased yield, resistance to pests, and resistance to environmental stresses (Hussin et al. 2022). Earlier sugarcane breeding technologies traditionally focused on enhancing the yield of bioethanol, with a focus on removing the challenges encountered by the recalcitrance of lignin to form effective saccharification of the lignocellulosic biomass for the production of bioethanol.

GE is thus becoming promising, providing precise tools that should eventually make possible modifications to traits of lignin content or facilitate biomass conversion with significantly greater efficiency. Early developments in genome editing to improve lignocellulosic ethanol conversion used TALEN-based gene editing for caffeic acid *O*-methyltransferase (COMT), which is essential for lignin synthesis. This strategy effectively decreased the amount of lignin, increased the saccharification efficiency, and improved the composition of the cell wall (Jung and Altpeter 2016; Kannan et al. 2018). ZFNs and TALENs are generally less efficient, more complex in design, and more likely to target other parts of the genome. On the other

Table 2.2 CRISPR- and TALEN-driven genetic modifications in sugarcane

Technology	Variety	Mutation frequency	Targeted gene	Function	Remark	References
TALEN	CP88–1762	7.8–99.1%	Caffeic acid *O*-methyltransferase (*COMT*)	Lignin production	Decreased lignin content	Jung and Altpeter (2016)
TALEN	CP88–1762	51.5–92.5%	*COMT*	Lignin production	Decreased lignin content	Kannan et al. (2018)
CRISPR	CP88–1762	4.8–83%	Magnesium chelatase (*MgCh*)	Chlorophyll biosynthesis	Reduced chlorophyll content	Eid et al. (2021)
CRISPR	CP88–1762	35–43%	Acetolactate synthase (*ALS*)	Branched amino acids biosynthesis	Resistance to nicosulfuron herbicide	Oz et al. (2021)
CRISPR	KK3	–	SoLIM (transcription factor)	Lignin biosynthesis	Reduced lignin content	Laksana et al. (2024)
CRISPR	ROC22	–	ScMTL (matrilineal)	Haploidy induction	Increased haploid induction rate	Guo et al. (2024)
CRISPR	CP88–1762	7.4–100%	Liguleless1 (*LG1*)	Regulation of leaf inclination angle	Improved biomass content	Brant et al. (2024)

hand, CRISPR provides an easier design, the ability to edit multiple alleles of a gene, increased precision, and significantly reduced costs to make it a far more accessible and reliable approach in genetic engineering (Zhang et al. 2018b).

DNA repair pathways such as nonhomologous end joining (NHEJ) and homology-directed repair (HDR) fix site-specific double-strand breaks (DSBs) caused by CRISPR technology. These repair mechanisms facilitate targeted mutagenesis through gene insertions or deletions, enabling precise gene editing (Xue and Greene 2021). A crucial component of chlorophyll biosynthesis, the magnesium chelatase gene (*MgCh*), was precisely coedited by means of the first successful application of CRISPR/Cas9 in sugarcane. This development highlighted the adaptability of CRISPR toward intricate genetic systems like polyploid sugarcane by offering a means of modifying photosynthesis at the canopy level (Eid et al. 2021). Multiplex allelic editing using CRISPR technology has also been used to create herbicide-resistant transgenic lines, which result in amino acid substitutions in the acetolactate synthase (*ALS*) gene (Oz et al. 2021). Genome-edited transgenic lines created using CRISPR to knock out SoLIM transcription factors (TFs), which are responsible for downregulating lignin biosynthesis genes, demonstrated improved lignocellulosic bioethanol production and enhanced saccharification efficiency (Laksana et al. 2024).

The manipulation of genetic pathways in sugarcane presents fascinating opportunities for optimizing plant architecture. A notable advancement involves the multiallelic editing of the Liguleless1 (*LG1*) gene, which is crucial for regulating leaf inclination angle. This targeted gene modification has enhanced leaf architecture, positively impacting biomass yield by improving key agronomic traits, such as leaf angle, tiller number, and internode length (Brant et al. 2024). Targeted mutagenesis of desired genes promises the development of improved sugarcane cultivars. Despite its potential benefits, genome editing is facing issues related to effective biosafety regulations, which is standing in its way to progress. However, one breakthrough area in genetically modified (GM) sugarcane release is CanaFlex 1 and CanaFlex 2, released in Brazil as genetically engineered varieties and classified as transgene-free. Such classification counts as an advanced milestone in innovative sugarcane cultivar development since regulatory concerns, mostly related to the perception of the public toward genetically modified organisms, are addressed.

2.2.6 RNA Interference (RNAi) in Sugarcane

RNA interference (RNAi) is an important biological process that controls posttranscriptional expression of specific sequences of genes through small noncoding RNAs (ncRNAs), including siRNAs and miRNAs (Matzke et al. 2001). The enzyme Dicer cleaves double-stranded RNA (dsRNA), which yields small ncRNAs. When the RNA-induced silencing complex (RISC) and Argonaute proteins help small ncRNAs pair up with their target mRNAs, they degrade and effectively regulate gene expression, resulting in gene silencing. Targeted gene manipulation through

RNAi comprises identifying the target gene, making the RNAi construct, introducing it into the host plant through genetic transformation, and assessing the resultant traits to evaluate the efficacy of gene silencing and its effects (Kamthan et al. 2015). The introduction of RNAi technology in sugarcane targeted the generation of transgenic lines with reduced lignin content for enhanced saccharification efficiency.

Downregulation of the *COMT* gene resulted in lower lignin content levels, making biomass conversion better in RNAi mutants than in wild-type plants (Jung et al. 2012; Jung et al. 2016). Additionally, RNA interference (RNAi) changed the expression of three important genes involved in lignin biosynthesis: *COMT*, ferulate 5-hydroxylase (*F5H*), and caffeoyl-CoA *O*-methyltransferase (*CCoAOMT*) in order to alter the lignin content and produce better lignocellulosic bioethanol (Bewg et al. 2016). RNAi-induced silencing of the *SacBAHD01* gene resulted in increased saccharification and enhanced bio-digestibility through reduction of ferulate content as a new approach for higher bioethanol quality (de Souza et al. 2019). RNAi has been efficiently employed to combat SCMV, a major pathogen in sugarcane, by designing hairpin constructs targeting viral coat protein genes. These constructs triggered RNA silencing pathways, leading to the generation of transgenic lines with enhanced viral resistance by effectively degrading the viral RNA, thereby limiting SCMV's impact on sugarcane yield (Aslam et al. 2018; Sawitri et al. 2024).

RNA silencing has been extensively developed for sugarcane to enhance resistance against various stresses such as drought (Selvi et al. 2021), salinity (Mazalmazraei et al. 2023), and pests (Wang et al. 2017). The regulation of genes involved in sugar metabolism has been linked to miRNAs' function in raising the amount of sucrose in sugarcane. MicroRNAs such as miR172, miR164, miR396, and miR169 are involved in controlling genes associated with sucrose metabolism, including those encoding enzymes like cell wall invertase, glucosidase, hexose carriers, sucrose transporters, UDP-sugar pyrophosphorylase, and soluble acid invertase (Wang et al. 2022). The development of miRNA databases, including MicroSUGAR, miRBase, and VIRmiRNA, and advancements in sugarcane genome sequencing can be further utilized to create superior sugarcane cultivars with other desired characteristics.

RNAi technology for gene silencing has already proven to have tremendous potential in enhancing the resilience and productivity of sugarcane. By targeting the key genes engaged in processes such as biosynthesis of lignin and resistance to pests and pathogens, RNAi can help improve various traits, resulting in varieties with better yield, saccharification efficiency, and stress tolerance. As RNAi technology continues to evolve alongside advances in genomics and bioinformatics, its integration into sugarcane breeding programs can offer new avenues for tackling the challenges posed by complex polyploid genomes. Ultimately, RNAi represents a powerful tool for achieving precise genetic modifications in sugarcane, fostering more sustainable agricultural practices and supporting global food and biofuel demands.

2.2.7 Genetic Transformation in Sugarcane

Sugarcane production has been hindered by various biotic (weeds, pests, and diseases) and abiotic factors (drought, salinity, cold), prompting genetic transformation to address these challenges and improve crop yields. Genetic transformation, which involves the transfer of desirable genes of interest, offers a powerful alternative to conventional breeding, effectively addressing the challenges posed by non-flowering nature and long development times in sugarcane. *Agrobacterium*-mediated transformation (AMT) utilizes *Agrobacterium* as a vector to facilitate the transfer of DNA into plant cells (Arencibia et al. 1998). Alternatively, the biolistic approach enables foreign DNA to be directly inserted into the plant genome (Bower and Birch 1992). Both techniques are now widely employed in sugarcane genetic transformation, allowing the development of elite varieties with desirable agricultural traits. Bower and Birch (1992) pioneered the first transgenic sugarcane plant using the biolistic method, which, although applicable to various hosts without a vector, presents challenges such as multiple transgene insertions, genomic rearrangements, and transgene silencing (Travella et al. 2005).

AMT has been widely adopted due to its advantage of fewer transgene insertions and reduced instability compared to other methods, making it an effective approach for sugarcane genetic modification. The first successful transgenic sugarcane developed through AMT was glufosinate-resistant, achieved by introducing the *bar* gene, which encodes phosphinothricin acetyltransferase, conferring resistance on glufosinate (Enríquez-Obregón et al. 1998). Several transgenic sugarcane lines have been successfully modified to be resistant to herbicides by introducing genes that confer resistance on glufosinate, such as *pat* (Snyman et al. 1998) and *bar* (Chowdhury and Vasil 1992; Gallo-Meagher and Irvine 1996; Falco et al. 2000). The *bar* gene encodes phosphinothricin acetyltransferase, which inactivates glufosinate, thereby protecting the plant from the herbicide toxic effects and effectively managing weed control. Similarly, glyphosate-tolerant transgenic lines utilizing the *EPSPS* (5-enol pyruvylshikimate-3-phosphate synthase) gene have been developed more recently to curb weed competition, with studies showing successful field applications (Nasir et al. 2014; Racedo et al. 2019). Transgenic sugarcane varieties expressing insecticidal proteins, such as lectins and protease inhibitors, effectively control pests by disrupting their growth, development, and reproduction.

These proteins impair the pests' digestive processes, offering an environmentally friendly alternative to chemical pesticides for sustainable pest management in sugarcane. Sugarcane borers are a significant threat to sugarcane production, driving the development of transgenic lines expressing insecticidal proteins such as cry1Ab, cry1Ac, and cry2a (Xu et al. 2008; Arvinth et al. 2010; Gao et al. 2018). Furthermore, transgenic sugarcane with genes for protease inhibitors such as aprotinin (Christy et al. 2009), skti (Falco and Silva-Filho 2003), and Vip3A proteins (Riaz et al. 2020) have demonstrated improved resistance to borers. Capsid proteins are expressed in several transgenic sugarcane lines to provide resistance against viral infections, specifically SCYLV (Gilbert et al. 2009; Zhu et al. 2011) and SCMV (Apriasti et al.

2018; Aslam et al. 2018). However, the development of sugarcane varieties resistant to both glyphosate and pests, such as cry1Ab-expressing lines for borer resistance, faced challenges, as they exhibited poor agronomic and industrial traits in field conditions (Wang et al. 2017). Transcription factors are vital in regulating gene expression, especially under abiotic stress conditions. Research highlights the critical role of transcription factors in enhancing stress resilience in sugarcane. Overexpression of *Arabidopsis* dehydration-responsive element binding 2A (DREB2A) has shown substantial effects in boosting stress tolerance (Reis et al. 2014), while the tomato ethylene response factor 1 (*TERF1*) gene from tomatoes has also demonstrated its potential to strengthen stress tolerance under various environmental conditions (Rahman et al. 2021).

These findings suggest that leveraging such transcription factors can significantly enhance crop resilience. Moreover, transgenic sugarcane lines have made strides in resisting abiotic stresses like salinity and cold. Salinity tolerance is achieved through the overexpression of the *P5CS* gene, which aids in proline synthesis, serving as an osmoprotectant to maintain cellular integrity under osmotic stress (Guerzoni et al. 2014). Cold tolerance improvements have been linked to genetic modifications, such as the overexpression of the *ipt* (isopentenyl-transferase) gene, enhancing root development and plant vigor (Belintani et al. 2012), and the *SoTUA* gene for α-tubulin (Chen et al. 2021).

While the commercial release of transgenic sugarcane lags behind other major crops like maize, soybean, and cotton, this delay is largely due to the intricate genome of sugarcane, which requires labor-intensive, costly transformation, and deregulation processes. The complexity of the sugarcane genome, combined with hurdles such as transgene instability and regulatory concerns, has posed significant challenges to releasing transgenic cultivars. Establishing robust transformation pipelines and continued refinement of these techniques, along with emerging technologies like CRISPR and TALEN, will ensure that sugarcane, like other major crops, benefits from the full spectrum of modern biotechnological advancements.

2.3 Multi-Omics in Sugarcane

Multi-omics integrates diverse high-throughput technologies like genomics, transcriptomics, proteomics, and metabolomics, providing a thorough understanding of complex biological systems. In the context of sugarcane, these approaches are vital due to their polyploid nature, complex genome structure, and significant economic importance for sugar and bioenergy production. By merging data from different omics layers, researchers can gain insights into molecular networks, which can significantly aid in sugarcane breeding programs and crop improvement efforts (Fig. 2.4).

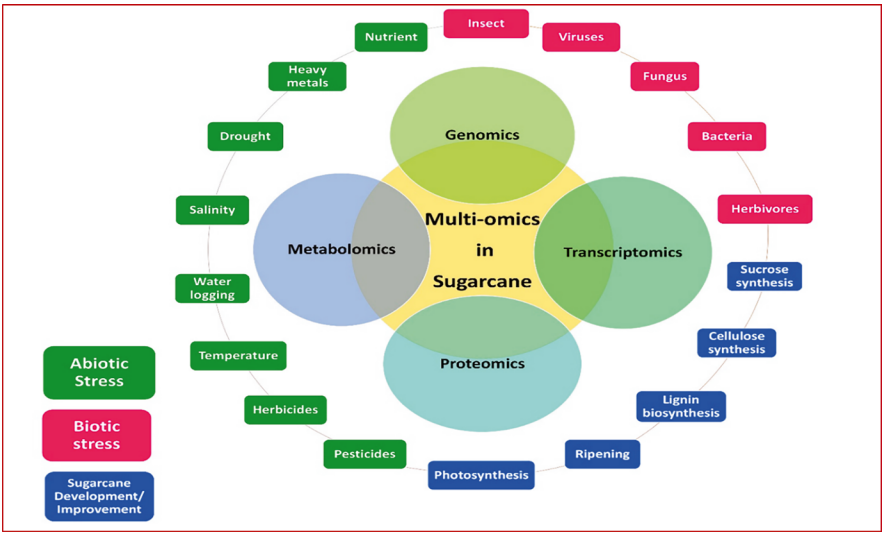

Fig. 2.4 Multi-omics approaches for sugarcane improvement

2.3.1 Sugarcane Genomics

The sugarcane genome, estimated to be approximately 10 gigabases (Gb), is significantly larger than that of the human genome (Souza et al. 2011; Zhang et al. 2012) and varies considerably across cultivars, ranging from 3.80 to 10.96 Gb (Berkman et al. 2014). Despite the genome's complexity, due to high polyploidy and extensive repetitive sequences, the identification of genes essential for characteristics like sugar content, disease resistance, and biomass yield has been made feasible by developments in sequencing technologies. These genomic insights are foundational for sugarcane improvement, especially for multi-omics approaches (Fig. 2.1). Genomic selection (GS) has revolutionized sugarcane breeding, enabling faster genetic gains and shorter breeding cycles (Meuwissen and Goddard 2001). Complex traits like cane yield, number of millable canes, single cane weight, and commercial cane sucrose, which are controlled by many small-effect genes, are challenging to improve using traditional MAS.

However, GS effectively captures these polygenic effects, making it a more precise tool for enhancing sugarcane productivity (Gouy et al. 2013; Crossa et al. 2017). One of the most challenging aspects of sugarcane genomics is its genome assembly, mainly due to its high heterozygosity and polyploidy. Sugarcane cultivars originated from two species, *S. officinarum* (930 Mb) and *S. spontaneum* (750 Mb), which differ significantly in genome size and structure (Meena et al. 2020). Recent advances, such as the autopolyploid *S. spontaneum* genome sequencing using haploid lines, have revealed key chromosomal rearrangements, including fission and translocation events that have reduced the number of chromosomes from 10 to 8.

Notably, these rearranged chromosomes contain 80% of the nucleotide-binding sites (NBSs) that encode the genes involved in disease resistance (Zhang et al. 2018a).

However, bacterial artificial chromosome (BAC) sequencing has been used to align sugarcane sequences with the *Sorghum* genome, allowing researchers to assemble high-quality sequences and predict more than 25,000 protein-coding genes, many of which show no collinearity with *Sorghum* (Garsmeur et al. 2018). These efforts have offered crucial insights into the genetic structure of sugarcane, helping breeders better understand chromosomal rearrangements and transposable elements that drive genome evolution and polyploidy.

Genomic approaches like GWASs have been essential in identifying marker–trait associations (MTAs) linked to key agronomic characters such as stalk number, plant height, and cane yield. GWASs have identified loci associated with resistance to diseases such as orange rust and SCYLV (Yang et al. 2019; Pimenta et al. 2021). These insights help breeders select for disease-resistant cultivars and manage breeding programs more effectively. Despite the ongoing challenges in assembling a complete reference genome for modern sugarcane, ongoing advancements in deep sequencing and transcriptomics are shedding light on allelic variations and gene expression patterns. By combining GS, GWASs, and functional genomics, researchers are better equipped to improve sugarcane yield, stress tolerance, and disease resistance, ultimately contributing to more sustainable and productive sugarcane cultivation.

2.3.2 Sugarcane Transcriptomics

Transcriptomics, the study of RNA transcripts, is crucial for understanding gene expression patterns in sugarcane across various conditions, such as developmental stages, stress responses, and key metabolic processes like sucrose metabolism and biomass production. These studies use techniques like expressed sequence tags (ESTs), probe hybridization arrays, and data from allied crops to identify critical genes. The Brazilian Sugarcane EST (SUCEST) project, a significant milestone, collected 237,965 ESTs from 37 cDNA libraries, which were clustered into 43,141 sugarcane-assembled sequences (SASs) (Vettore et al. 2001). These efforts have resulted in the most comprehensive sugarcane gene index to date (v3.0), comprising 282,683 ESTs, 499 cDNA sequences, and 121,342 unigenes. However, despite such extensive efforts, about 10,000 coding genes in sugarcane remain unidentified, posing a challenge to fully understanding the genome (Xu et al. 2018). Transcriptomics has enabled researchers to validate gene expression linked to key traits such as photosynthesis, ripening, leaf abscission, and lignin and cellulose biosynthesis (Shabbir et al. 2021).

It has also deepened our understanding of the molecular mechanisms governing plant resistance to biotic and abiotic stress factors (Li et al. 2023a, b; Puri et al. 2023). Furthermore, transcriptomic techniques offer valuable resources for genetic improvements by identifying stress-responsive TFs like zinc-finger, AP2/DREBP,

bZIP, MYB, and WRKY (Song et al. 2011). For instance, research on red rot caused by *Colletotrichum falcatum* revealed early induction of transcription factors such as NAC, WRKY, MYB, TLP, and bZIP, which coordinate defense mechanisms (Nishiyama Jr et al. 2014). Differentially expressed genes (DEGs) responsible for oxidative stress response, ethylene biosynthesis, ethylene and jasmonic acid responsive pattern recognition receptors (PRRs), cell wall fortification, oxidative bursts, and cell wall fortification were found in studies on red stripe disease caused by *Acidovorax avenae* subsp. avenae (Santa Brigida et al. 2016; Chu et al. 2019). Abiotic stressors like drought have been shown to affect sugarcane varieties differently through transcriptomic studies. This is especially true for drought-tolerant cultivars like SP81–3250, where genes encoding ascorbate peroxidase, coenzyme A ligase, and aquaporin are activated. Conversely, these genes remain inactive in drought-sensitive cultivars. Stress-induced genes, such as those encoding receptor-like kinases (RLKs), ACC oxidase, and bHLH transcription factors, play a role in drought-responsive defense and other abiotic stress conditions (Ali et al. 2019b).

Drought-tolerant varieties show increased expression of genes linked to antioxidant production, secondary metabolites, water retention, and resistance to oxidative and osmotic stress. In contrast, drought-sensitive varieties show downregulation of genes involved in the Calvin cycle, carbon fixation, and photosynthesis under water deficit conditions (Nawae et al. 2020). Metabolic processes critical to sugarcane's growth have also been explored through transcriptomics, identifying the genes responsible for oxidative detoxification, root growth, fatty acid biosynthesis, and glycolysis. The drought-tolerant species *Saccharum narenga* further revealed 3,389 DEGs involved in hormone signaling and other metabolic pathways (Liu et al. 2018).

Recent technological advances in transcriptomics have addressed some challenges in sugarcane research.

Despite its widespread use, high-throughput RNA sequencing (RNA-Seq) produces short reads that necessitate substantial computational assemblies and lower the accuracy of gene models. Single-molecule long-read sequencing technologies like Pacific Biosciences' Iso-Seq offer a completer and more accurate alternative, allowing for better prediction of gene models (Wang et al. 2016). These transcriptomic methods have shown that stress-tolerant sugarcane varieties have significantly higher levels of several genes, such as those encoding ascorbate peroxidase 3 (*APX3*), coenzyme A ligases, E3 SUMO protein ligase SIZ2, MYB TFs, and aquaporins (Nishiyama Jr et al. 2014; Guo et al. 2017). Despite these advancements, one of the significant hurdles in sugarcane transcriptomics is the absence of an entirely accurate reference genome. Consequently, the *Sorghum bicolor* genome, which shares 95% homology with sugarcane in genic regions, is often used as a reference in sugarcane transcriptome studies. Sugarcane genotypes vary genetically; approximately 47% of sugarcane unigenes match *Sorghum bicolor* proteins, but only 2% match the sugarcane hybrid cultivar R570 (Xu et al. 2018). Despite significant strides in identifying the genes that influence sugarcane's growth and stress responses, more extensive research is needed to discover the remaining coding genes and develop cultivars with improved stress resistance and productivity.

2.3.3 Sugarcane Proteomics

Proteomic analysis is essential for deciphering the intricate biological mechanisms in sugarcane, particularly under various environmental stresses. Advanced techniques such as two-dimensional gel electrophoresis (2-DE), mass spectrometry (MS), and isobaric tags for relative and absolute quantitation (iTRAQ) have been extensively employed to explore protein expression and post-translational modifications (PTMs) in sugarcane when subjected to both biotic and abiotic stress conditions. Through these methods, researchers have successfully identified differentially expressed proteins (DEPs) that play vital roles in crucial biological processes, including photosynthesis, signal transduction, defense mechanisms, and energy metabolism. For instance, studies have highlighted the importance of proteins associated with the pathways of reactive oxygen species (ROS), abscisic acid (ABA), and phenylpropanoid metabolism, which are instrumental in enhancing resistance to pathogens such as *Sporisorium scitamineum* and *Colletotrichum falcatum* (Que et al. 2011; Shabbir et al. 2021). Furthermore, quantitative proteomic approaches have revealed significant insights into the defense mechanisms against bacterial infections, particularly those caused by *Xanthomonas albilineans* (Meng et al. 2020). These findings emphasize the pivotal role of specific proteins in providing resistance and enhancing overall plant health.

In addition to the impact of biotic stress, proteomics has been crucial in investigating sugarcane's response to drought and salinity. Research has demonstrated that sugarcane cultivars resistant to drought and susceptible to it differ in the expression of proteins involved in photosynthesis, signal transduction, and cell wall metabolism. Proteomic analyses have uncovered key transcription factors from families such as NAC and MYB, facilitating the plant's adaptive responses to drought stress (Song et al. 2011). Similarly, under salt stress, research has identified proteins upregulated in tolerant sugarcane varieties, specifically those involved in membrane stabilization, energy metabolism, and ROS metabolism (Pacheco et al. 2013). Proteomic analysis of genotypes with contrasting tolerance to saline soil have revealed significant differences in protein expression between tolerant and sensitive varieties. Lipid-metabolizing enzymes and Gly-Asp-Ser-Leu (GDSL) motif lipases, which are linked to abiotic stress defense mechanisms, were three times more abundant in the salt-tolerant genotype.

Furthermore, the tolerant genotype had twice as much lipoxygenase and type III chlorophyll a/b-binding protein compared to the sensitive genotype. Phosphoenolpyruvate carboxylase, a crucial enzyme for C4 photosynthesis, and other chloroplast enzymes were notably downregulated in the susceptible genotype (Chiconato et al. 2021). The proteomic data indicate that these proteins contribute significantly to maintaining cellular integrity and function under stress conditions. These comprehensive proteomic studies enhance our understanding of the molecular basis of sugarcane's resilience to environmental challenges and provide a foundation for improving crop productivity and developing stress-resistant cultivars. They seem to hold plenty of promise for improving sugarcane production in the face

of stressors like climate change by combining proteomic insights with conventional breeding and genetic engineering techniques.

2.3.4 Sugarcane Metabolomics

Metabolomics is a comprehensive approach that links physiological and phenotypic changes in plants to external stimuli by analyzing low-molecular-weight compounds. Although sugarcane metabolomics is still in its infancy, it provides significant insights into sugarcane biology, mainly through assessing metabolite levels, especially carbohydrates such as fructose, glucose, raffinose, sucrose, and inositol. These metabolites are characterized using a variety of analytical methods, such as gas chromatography (GC), nuclear magnetic resonance (NMR), and mass spectrometry (MS). Understanding the relationship between metabolomics, transcriptomics, and proteomics is critical for advancing our knowledge of sugarcane breeding and developing cultivars with enhanced resistance to biotic and abiotic stress conditions (Hao et al. 2018; Shabbir et al. 2021). Recent metabolomic studies have focused on the interactions between sugarcane and pathogens to identify potential markers for developing resistant cultivars. For example, studies on sugarcane plants infected with SCYLV showed notable alterations in carbohydrate levels, suggesting decreased export of assimilate from infected leaves (Lehrer et al. 2007).

Similar studies have shown that under the yellow canopy syndrome (YCS), infected plants exhibit increased sucrose content alongside reduced stomatal conductance and photosynthetic rates (Marquardt et al. 2016). Moreover, specific metabolites like Apigenin 7-O-(600-O-acetylglucoside) have been identified as potential markers for healthy plants (Schaker et al. 2017). Metabolomics has provided insights into how sugarcane reacts to abiotic stressors like drought, salinity, and pathogen interactions. The application of proline, for example, has been shown to mitigate heat stress effects by enhancing osmolyte production and stabilizing cellular processes (Rasheed et al. 2011). The importance of metabolomics extends to understanding the mechanisms of sugar accumulation in sugarcane, which is essential for enhancing crop yields. Research has established a connection between metabolite profiles and the key genes involved in sucrose metabolism, which is influenced by various factors, including sink demand and genetic regulation. Techniques like GC–MS have been instrumental in profiling metabolic changes during sugarcane development, revealing correlations between sucrose accumulation and metabolite abundance (Bosch et al. 2003; Ali et al. 2019b). Such insights inform breeding programs aimed at developing stress-tolerant sugarcane genotypes and improving understanding of the genetic basis of sugarcane productivity. The continued exploration of sugarcane metabolomics promises to uncover valuable information that can enhance the resilience and efficiency of sugarcane cultivation in the face of environmental challenges.

2.4 Concluding Perspectives and Future Outlook on Sugarcane Breeding

While benefiting from numerous advanced technologies (Fig. 2.1), sugarcane breeding still faces severe constraints that hamper speedy genetic improvement. One major challenge is its long crop cycle, which takes approximately 12–14 years to develop a new variety using conventional breeding methods. This breeding primarily depends on phenotypic selection, which involves mass and family selection across long crop cycles, with extensive field evaluations. Unfortunately, low narrow-sense heritability and high nonadditive genetic variance contribute to the low genetic gain for cane yield (Barbosa et al. 2012). Additionally, sugarcane flowering is asynchronous, which complicates breeding efforts. Although speed breeding offers a promising approach by accelerating plant development and cycling generations under controlled conditions (Watson et al. 2018), it is still in developmental stages. Its effectiveness under both controlled and field conditions remains obscure at present. However, combining speed breeding with GS into a SpeedGS approach could address some of these limitations by providing rapid breeding cycles with short generation times and synchronizing flowering through photoperiod manipulation. That said, the applicability of SpeedGS in sugarcane is still largely unexplored, perhaps because of the crop's considerable height and extensive growth habits, making it complicated to accommodate sugarcane within typical controlled environments and glasshouses.

The widespread use of a few elite varieties, or "nobilization" of canes, has decreased genetic diversity and increased the susceptibility of cultivars to pests and diseases, thereby slowing down progress toward yield and biomass production. Techniques such as mutation breeding and genome editing offer potential solutions by introducing genetic variability and preventing stagnation in sugarcane breeding programs. Furthermore, the complex polyploid nature and large genome size of sugarcane pose additional challenges for QTL exploration and MAS. However, the assembly of the complete polyploid reference genome of the sugarcane cultivar R570, which comprises 8.7 billion base pairs (Healey et al. 2024), has opened new avenues for molecular breeding and transgenic approaches in sugarcane. The complete genome sequence provides a robust framework for exploring genetic complexity and leveraging genome editing tools in sugarcane. In addition, plant epigenomics is emerging as a powerful tool for understanding gene regulation mechanisms such as histone modifications, DNA methylation, noncoding RNAs, and chromatin remodeling (Perrone and Martinelli 2020). These insights are crucial for understanding the molecular responses of sugarcane to a wide range of stressors.

They can potentially open avenues for future developments in stress tolerance enhancement and increased crop resilience. The amalgamation of the methodologies presented in this chapter, integrated with more novel computational techniques like machine and deep learning methods, can be highly instrumental in accelerating sugarcane breeding. High-throughput genotyping and phenotyping will enhance precision and significantly enhance biomass yield, stress tolerance, and overall

genetic gain. These advancements may influence global biofuel policies and sugarcane production strategies. The practical establishment and application of emerging techniques will require collaboration among breeders, molecular biologists, geneticists, and computational specialists to bring these innovations into action strategies for variety improvement in the future.

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Chapter 3
Utilization of Sugarcane Germplasm for Breeding Climate-Resilient Varieties: A Critical Research Topic

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Abstract Global warming is becoming an even greater challenge for sugarcane, a key raw material for producing sugar and ethanol. To ensure sustainable production, it is essential to develop climate-resistant cultivars. This chapter highlights the importance of using sugarcane germplasm to breed cultivars resilient to various climatic conditions. It explains the value of germplasm diversity and its role as the genetic foundation for breeding program. Various germplasm assessment techniques, including phenotypic and molecular techniques, are discussed. This chapter also covers the application of genomic technologies to identify genes and alleles linked to climate resilience. Different breeding strategies, such as conventional breeding, marker-assisted selection, and genetic engineering, are explored for developing climate-resilient varieties. Overall, this chapter provides a comprehensive overview of how sugarcane germplasm can be utilized to breed sustainable, climate-resilient cultivars in the face of climate change.

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3.1 Introduction

Climate change is a major global issue, involving significant and long-term changes in climatic patterns, mainly caused by human activities like industrialization, deforestation, and the burning fossil fuels (Kumari et al. 2022, 2023; Jagadesh et al. 2024). It includes changes in temperature, precipitation patterns, the frequency of extreme weather events, rising sea levels, and shifts in atmospheric composition (Devi et al. 2024a; Kumar and Singh 2024a; Sharma et al. 2024). Climate change has widespread impacts on the environment, biodiversity, and agriculture, and sugarcane is one of the crops most vulnerable to these changes (Verma et al. 2019a, b). Sugarcane (*Saccharum* spp.) is a commercial important crop, well adapted to warm and rainy regions and used for producing sugar, ethanol, and bioenergy. However, climate variability presents major challenges for sugarcane production (Singh et al. 2019; Verma et al. 2024a, b). Sugarcane requires specific climatic conditions, viz., warm, humid climates with annual rainfall between 1200 and 1500 mm and is highly sensitive to changes in temperature, water availability and other environmental factors (Song et al. 2022; Malviya et al. 2020, 2022).

Rising global temperatures increase evapotranspiration rates, leading to greater water demand for crops (Chen et al. 2020, 2024). Water scarcity is already considered a limiting factor in certain regions of India, Brazil, and parts of Africa, and higher temperatures are likely to worsen water stress, reducing sugarcane yields (Verma et al. 2020a, b, 2021a; Wu et al. 2022). Heat waves shorten the sugar accumulation period, impacting final yields, as sugarcane typically takes 12–18 months to mature (Devi et al. 2024b; Kumar et al. 2024a, b). Increasing temperatures are also expected to lower sugarcane juice quality, affecting sugar and ethanol production (Kumar and Singh 2024b; Sharma et al. 2024).

Besides temperature, erratic rainfall patterns present significant challenges (Singh et al. 2021; Verma et al. 2021b, c; Krishna et al. 2022). Excessive rainfall causes waterlogging, reducing oxygen availability to roots and stunting plant growth, while drought inhibits photosynthesis, slowing development. Future predictions suggest more frequent extreme weather events such as hurricanes and cyclones, floods, and droughts (Verma et al. 2020c, d; Singh et al. 2021, b; Guo et al. 2022). Rising sea levels will increase soil salinity along the coastal regions further stressing sugarcane production. Salinity reduces germination growth, and yield, making climate-resilient breeding efforts critical (Verma et al. 2023a, b, 2024c, d; Bhatt et al. 2024). To address these challenges, one of the strategies is perhaps to breed sugarcane varieties that are not susceptible to the effects of climate change. Hence, the primary goal of climate resilience breeding is to develop varieties of sugarcane

that are less sensitive to heat, drought, water logging risks, and salinity (Kumar et al. 2024a; Pandey et al. 2024; Sharma et al. 2024; Huang et al. 2024).

Understanding sugarcane genetics is crucial for breeding climate-resistant cultivars (Verma et al. 2020e, f; Anas et al. 2021a, b; Oliveira et al. 2022). However, breeding sugarcane is complex due to its high degree of polymorphism and polyploidy. Traditional breeding methods involve crossing various lines to enhance yield, sugar content, and disease and pest resistance. In the wake of climate change, breeding programs must prioritize tolerance to abiotic stresses such as heat, drought, and salinity (Sharma et al. 2021, 2022). Germplasm resources, viz., seeds, pollen, and tissues play a vital role in developing new sugarcane varieties, which are used in breeding programs to create new crops. Germplasm collections preserve genetic diversity, providing traits like stress tolerance essential for breeding climate-resilient crops (Afghan et al. 2024; Verma et al. 2021f; Singh et al. 2022; Liang et al. 2024; Chen et al. 2024).

Presently, gene banks and botanical gardens preserve germplasm to be used for breeding and research activities in the future. Wild sugarcane relatives, landraces, and cultivated varieties are all components of the sugarcane germplasm repository (Singh et al. 2021, 2023). Wild relatives such as *Saccharum spontaneum* and *Saccharum robustum* offer valuable traits like drought and salt tolerance (Verma et al. 2021d, e; Guo et al. 2021; Xu et al. 2022). Sampling and assessing germplasm diversity is the first step in breeding programs (Dey et al. 2024). These efforts will be crucial for developing robust sugarcane cultivars capable of thriving in changing climates.

3.2 Sugarcane Germplasm Diversity

3.2.1 Importance of Germplasm Diversity in Breeding Programs

In plant breeding, germplasm diversity is irreplaceable. Genetic differences in a species develop over time due to natural selection and environmental pressures captured in various alleles, genes, and trait combinations. Enhancing cane yield and developing varieties that can withstand environmental factors such as drought, heat and salt stress, and resistance to diseases and pests require germplasm diversity. Breeding programs rely on broad genetic base to meet the increasing demands of modern farming systems. As climate change adversely impacts many crops, including sugarcane, maintaining genetic diversity is critical. This diversity allows breeders to select traits that enable sugarcane to adapt to changing growing seasons, limited water availability, or higher temperatures. Sugarcane germplasm is highly heterogeneous, providing opportunities to combine traits that enhance sugar yield, disease resistance, and biomass quality important for both sugar and ethanol production (Deng et al. 2023).

Climate change has added unpredictability to the growth of polyploid crops like sugarcane. To overcome these challenges, breeders must utilize genetic materials that offer favorable alleles. Studies have shown that traits such as osmotic adjustment, root development, and water usage efficiency present within diverse germplasm collections are valuable for breeding drought-tolerant varieties (Malviya et al. 2021; Li et al. 2023). However, breeding must be a continuous process, as pests and diseases evolve rapidly, particularly under monoculture systems. Sugarcane remains vulnerable to diseases such as smut, rust, and mosaic virus, and without resistant varieties, these diseases could cause severe losses (Tian et al. 2023; Verma et al. 2024c). Access to diverse germplasm makes it easier to find resistance genes for developing improved cultivars. Without such genetic resources, crops become more vulnerable to environmental stresses and production failures (Kumar and Raut 2024; Sharma and Kumar 2024).

3.2.2 Sources of Sugarcane Germplasm

Sugarcane breeding programs can obtain their germplasm mainly from three sources: wild relatives, landraces, and cultivated varieties. Wild relatives provide important genetic traits, especially for stress tolerance, while landraces and cultivated type contribute resilience and agronomic improvements.

3.2.2.1 Wild Relatives

Wild relatives such as *Saccharum spontaneum*, *Saccharum robustum*, and other Poaceae family members are crucial reservoirs of genetic diversity. These species, adapted to harsh environments, offer valuable traits like drought and salinity tolerance. Wild relatives also possess a rich pool of genes that confer resistance to various diseases. These species are often exposed to diverse pathogens in their natural habitats, which has led them to develop strong immunity over time. Compared to commercial sugarcane varieties, wild relatives generally contain a higher number of resistance genes. Therefore, breeders can introduce these valuable resistance traits into cultivated sugarcane through targeted breeding (Kumar 2024a, b, c).

Moreover, wild relatives exhibit greater genetic variation than cultivated types, making them an important source of novel traits that are not yet part of the existing sugarcane gene pool. However, utilizing wild relatives in breeding programs presents certain challenges. The process of hybridizing wild and cultivated sugarcane is often difficult due to significant genetic divergence between them. In many cases, wild species must be first crossed with intermediate germplasm or repeatedly backcrossed with cultivated lines to transfer desirable traits without compromising the adaptability or agronomic performance of the final variety (Devi et al. 2024b, c; Kumar et al. 2024c; Saurabh et al. 2024).

3.2.2.2 Landraces

Cultivars that growers have been cultivating and selecting for generations and that remain adapted to specific local regions are called landraces. Such varieties often possess unique attributes shaped by traditional farming practices and local environmental conditions. Even though landraces generally produce lower yields compared to commercial cultivars, they exhibit important resilience traits, such as resistance to local pests and diseases and tolerance to environmental stresses (Verma et al. 2022a). In addition, both natural and human-driven (artificial) selection have contributed to the development of landraces, making them a valuable reservoir of genetic diversity.

Some of these traits, which are closely linked to the climatic conditions and farming systems of their regions of origin, are introduced into modern sugarcane varieties through landraces in breeding programs. For example, a landrace that has evolved in a region with limited water availability over many generations may show leaner growth, higher water-use efficiency, and improved drought tolerance (Joshi et al. 2022, 2023; Bhatt et al. 2023a, b). These landraces provide a genetic foundation for developing new cultivars suited to agriculture in climate-vulnerable areas (Jagadesh et al. 2024; Kumar and Dey 2024; Raj et al. 2024; Srivastav et al. 2024).

3.2.2.3 Cultivated Varieties

Selective breeding programs have resulted in the development of modern sugarcane cultivars with desirable traits such as disease resistance, high sugar content, and other economically valuable characteristics. While these modern varieties exhibit less genetic variation compared to wild relatives and landraces, their genetic backgrounds still play an essential role in ongoing breeding efforts. One of the key challenges for breeders is to maintain the high yield of modern cultivars while also introducing new traits that improve their resilience to environmental stresses. To achieve this, breeders often cross high-performing cultivated varieties with genetically diverse landraces or wild relatives to incorporate qualitative traits such as stress tolerance. For example, a high-yielding cultivar may be crossed with a low-yielding wild type that exhibits strong drought tolerance. The goal is to develop a new hybrid that combines the stress-resilience of the wild parent with the high productivity of the cultivated variety. By doing so, breeders aim to integrate the best traits from both parents into a single, climate-resilient cultivar (Kumar et al. 2024c).

3.2.3 Role of Germplasm Collections and Conservation Efforts

It can, therefore, be concluded that germplasm collections are fundamental to the preservation and utilization of sugarcane diversity. These collections, commonly referred to as gene banks, contain samples representing a wide range of sugarcane

types, including wild relatives, landraces, and improved cultivars (Kumar and Pathak 2019; Kumar and Naik 2020). In essence, to ensure the continuity and progress of agricultural innovations, breeders must have access to a well-maintained reservoir of genetic material, underscoring the vital role of germplasm conservation (Kumar et al. 2020; Zheng et al. 2023).

3.2.3.1 Conservation of Genetic Diversity

Thus, germplasm collections play a crucial role in conserving natural genetic variation, with their primary function being the maintenance of sugarcane's genetic diversity. In situ populations of wild relatives and traditional landraces are increasingly threatened by habitat destruction, climate change, and the decline of traditional agricultural practices. Without appropriate conservation efforts, these valuable genetic resources are at risk of extinction. To prevent such losses, germplasm repositories preserve seeds, pollen, or tissue cultures under controlled conditions. Another key goal is to ensure the long-term retention of genetic diversity within collections. If a gene bank maintains only a limited number of accessions, its genetic richness may diminish over time, potentially leading to the loss of rare alleles. To mitigate this risk, gene banks should periodically replenish their collections and ensure they include a broad representation of genetic diversity (Afghan et al. 2024).

3.2.3.2 Facilitating Breeding Programs

Because germplasm collections serve as repositories and sources of genetic material, modern breeding programs are heavily dependent on their availability. These collections play a central role in pre-breeding research, where breeders seek critical traits such as high sugar content, drought tolerance, and disease resistance. Global germplasm repositories enable breeders to identify specific accessions with desired traits, which can then serve as valuable parental material for future breeding efforts. The effective utilization and conservation of germplasm require strong international collaboration, as sugarcane is cultivated in a wide range of agro-climatic zones (Kumar and Mistri 2020; Aley et al. 2021). Thus, acquiring germplasm from diverse geographical regions is essential to develop climate-resilient sugarcane varieties. Notable international efforts in sugarcane germplasm conservation include Brazil's World Collection of Sugarcane and Related Grasses (WCSRG) and Florida's International Sugarcane Germplasm Collection (ISGC). These collections maintain a broad spectrum of genetic diversity to support current and future breeding programs (Kumar et al. 2011, Chakraborty et al. 2021; Das et al. 2022; Song et al. 2021, Saini et al. 2023).

3.2.3.3 Conservation in the Context of Climate Change

Germplasm conservation strategies must evolve to ensure that the genetic diversity stored in gene banks remains relevant and useful to future breeding challenges. To achieve this, the representation of accessions from regions likely to face significant climate change impacts should be expanded and prioritized.

3.3 Germplasm Characterization and Evaluation

Identifying and evaluating germplasm resources is the foundational step in utilizing genetic diversity to improve crop yields and develop climate-resilient varieties through breeding. Germplasm represents the full spectrum of genetic variation within a species, and researchers analyze both phenotypic and genotypic traits to uncover variability and assess the potential of different accessions. This step is essential for incorporating key traits such as heat tolerance, disease resistance, and drought tolerance into breeding programs. To maximize the utility of germplasm collections, it is now imperative to generate both phenotypic and genotypic data, offering a more comprehensive understanding of genetic diversity (Kumar and Dwivedi 2018; Xu et al. 2024). As climate change continues to pose significant challenges, modern breeding approaches must be strategic, data-driven, and goal-oriented. This chapter emphasizes the importance of integrating phenotypic and genotypic data, along with the use of molecular markers and genomic tools, to effectively characterize and utilize germplasm in contemporary breeding efforts.

3.3.1 Phenotypic Evaluation of Germplasm

Phenotypic evaluation involves assessing germplasm based on observable traits and characteristics under diverse environmental conditions. This approach enables breeders to identify accessions that exhibit desirable features such as stress tolerance, pest and disease resistance, and high yield potential. The phenotypic characterization process quantifies key agronomic traits, including biomass, plant height, root architecture, leaf area, and overall yield. Such evaluations are typically conducted either in controlled environments like phytotrons or directly in field conditions to ensure reliable trait expression (Reddy et al. 2022).

3.3.1.1 Drought Tolerance

Drought tolerance is a key trait targeted in sugarcane breeding programs, especially in light of the increasing frequency of water scarcity due to climate change. As sugarcane is a water-intensive crop, there is a growing need to develop varieties capable

of maintaining productivity under water-deficient conditions. Phenotypic evaluation of drought tolerance involves growing plants under controlled low-water environments and assessing their survival, growth performance, and yield. Typical traits associated with drought tolerance include deep and extensive root systems, which allow plants to access moisture from deeper soil layers during drought periods. Water-use efficiency, defined as the biomass or yield produced per unit of water consumed, is a critical parameter. Additional physiological traits used to assess drought tolerance include stomatal conductance, transpiration rate, and photosynthetic rate. One important adaptive mechanism under water stress is osmotic adjustment, where plants accumulate solutes to maintain cell turgor pressure. In the context of water-stressed cropping systems (WSCS), the development of drought-resistant sugarcane varieties is essential for ensuring sustainable production (Solanki et al. 2023; Malviya et al. 2024). Based on these observations, it is evident that phenotypic screening is an effective method for identifying germplasm accessions with drought-adaptive traits (Dwivedi and Kumar 2011; Pankaj et al. 2012a, b; Kumar and Dwivedi 2020; Dey et al. 2023c).

3.3.1.2 Heat Stress Tolerance

Heat stress tolerance is an increasingly important trait in sugarcane breeding, especially in the context of rising global temperatures. As climate change intensifies, breeders must prioritize the development of cultivars capable of withstanding elevated heat levels.

Phenotypic assessment of heat stress tolerance involves exposing plants to high-temperature conditions and evaluating their responses in terms of physiological and reproductive performance (Linnenluecke et al. 2018). Key traits assessed during heat tolerance screening include:

- Photosynthetic activity: Heat stress impairs the photosynthetic machinery. Heat-tolerant genotypes maintain photosynthetic function under elevated temperatures.
- Leaf temperature regulation: Effective transpirational cooling and other leaf-based mechanisms serve as indicators of thermal regulation in heat-tolerant plants.
- Membrane stability: Heat stress can damage cellular membranes, so membrane stability tests are used to identify accessions capable of maintaining integrity under stress.

A practical breeding strategy for heat resilience involves selecting germplasm that sustains growth, development, and reproductive success under high-temperature conditions.

3.3.1.3 Disease Resistance

Disease resistance is a critical phenotypic trait evaluated in sugarcane breeding programs, as sugarcane is highly susceptible to a wide range of fungal, bacterial, and viral pathogens. To screen germplasm for resistance, pathogens are either artificially introduced in controlled environments, such as greenhouses, or allowed to infect naturally under field conditions (Naik and Kumar 2020; Kumar et al. 2022a).

Some of the major diseases targeted in sugarcane breeding include:

- Smut (*Sporisorium scitamineum*): This fungal disease affects sugarcane stems and reduces yield. In resistant varieties, whether developed naturally or through artificial selection, there is a noticeable reduction in disease incidence and severity.
- Rusts (*Puccinia melanocephala* and *Puccinia kuehnii*): These cause brown rust and orange rust, respectively. Resistant germplasm displays fewer pustules, longer incubation periods, and lower disease severity.
- Sugarcane mosaic virus (SCMV): This viral disease disrupts photosynthetic efficiency and is identified through phenotypic traits such as mosaic patterns on leaves, stunted growth, and yield reduction.

Phenotypic screening enables breeders to identify accessions with disease resistance traits, which is crucial for minimizing yield losses and maintaining crop productivity (Kumar et al. 2018, 2021, b; Kumari et al. 2022; Hidangmayum et al. 2023; Sharma et al. 2023b).

3.3.2 Molecular Characterization Using DNA Markers and Genomic Tools

Molecular characterization provides insights into the genetic basis of traits, complementing phenotypic evaluation, which focuses on observable characteristics. Using genomic technologies and DNA markers, researchers can identify genetic variation within germplasm, enabling the mapping of key traits and facilitating faster and more targeted breeding through genomic selection (GS) and marker-assisted selection (MAS).

3.3.2.1 DNA Markers

Conversely, a DNA profile represents a specific combination of DNA markers associated with particular traits or genomic regions. These profiles are used to evaluate genetic variation among germplasm accessions through detailed accession-level analysis. In sugarcane breeding, several types of DNA markers are routinely used, including:

- Simple sequence repeats (SSRs): Also known as microsatellites, SSRs are short, repetitive DNA sequences that are highly polymorphic. Their variability makes them valuable tools for assessing genetic diversity.
- Single nucleotide polymorphisms (SNPs): SNPs represent single base-pair variations in DNA sequences and occur throughout the genome. They serve as precise markers for genetic mapping and association studies.
- Amplified fragment length polymorphisms (AFLPs): AFLPs detect differences in DNA sequences by amplifying specific genomic fragments. They are commonly used in linkage mapping and genetic diversity analysis.

DNA markers play a crucial role in identifying quantitative trait loci (QTLs) associated with important agronomic traits such as heat tolerance, disease resistance, and drought tolerance. Once QTLs are mapped, marker-assisted selection (MAS) can be applied to rapidly incorporate desired traits into new sugarcane varieties (Dey et al. 2023a; Afghan et al. 2024).

3.3.2.2 Genomic Tools

Modern genomic tools such as genomic selection (GS) and genome-wide association studies (GWAS) have become essential components of sugarcane breeding, complementing the use of conventional DNA markers (Pathak et al. 2017). These approaches allow for the genome-wide analysis of germplasm and enable the prediction of agronomic performance based on genetic profiles.

- Genome-wide association studies (GWAS): GWAS aims to identify associations between genetic variants and phenotypic traits. The diverse genetic base of germplasm collections makes them ideal for GWAS, which can locate specific genomic regions controlling complex traits, such as disease resistance and drought tolerance. This information is highly beneficial to breeders as it pinpoints target loci for selection in breeding programs.
- Genomic selection (GS): GS focuses on predicting the breeding value of an individual based on its genomic data. Unlike traditional breeding, which relies primarily on phenotypic evaluation, GS employs statistical models that integrate both genotypic and phenotypic information to forecast performance (Kumar et al. 2019; Dey et al. 2023b). This approach allows the development of improved cultivars with reduced dependency on extensive field trials.

Given that sugarcane is a polyploid crop with a highly complex genome, the application of advanced genomic tools is particularly well suited for its improvement. These technologies help breeders better understand genetic architecture and select optimal germplasm accessions for targeted trait improvement (Kumar et al. 2013, 2018, Singh et al. 2021; Sharma et al. 2023c).

3.3.3 Linking Phenotypic and Genotypic Data for Effective Germplasm Utilization

To maximize the effectiveness of germplasm in breeding programs, it is essential to integrate phenotypic and genotypic data. Phenotypic evaluation reflects how a plant performs under specific environmental conditions, providing insight into the expression of genetic traits in real-world settings. In contrast, genotypic data reveals the underlying genetic mechanisms and potential of a plant, independent of environmental influence. Combining these two data sources allows breeders to better understand the genetic architecture of key traits, enabling more accurate selection and decision-making in breeding programs.

3.3.3.1 Marker-Trait Associations

As previously mentioned, integrating phenotypic and genotypic data is crucial for identifying marker-trait associations. Establishing a link between genetic markers and observable traits allows breeders to determine which genomic regions govern important characteristics such as heat stress tolerance, disease resistance, and drought tolerance. Once such associations are identified, DNA markers become valuable tools for tracking the inheritance of desirable traits within breeding populations, enabling the efficient selection of individuals exhibiting these traits.

3.3.3.2 Precision Breeding

This approach becomes especially powerful when both phenotypic and genotypic data are applied in precision breeding to select optimal germplasm accessions for breeding programs. For example, a breeder can use a genetic marker in linkage disequilibrium with a trait of interest—such as drought tolerance—to identify plants carrying the favorable allele, even if they have not been exposed to drought stress. This strategy accelerates the development of new varieties while significantly reducing the time and resources required for field trials.

3.3.3.3 Enhancing Genetic Gain

Linking phenotypic and genotypic data enables breeders to accelerate genetic gain (GA)—defined as the improvement in trait performance across successive generations. Traditional phenotypic selection alone often results in slower genetic progress, as it is influenced by environmental variation and requires multiple growing seasons for evaluation. By incorporating genotypic data, breeders can identify and select individuals carrying favorable alleles for target traits, such as disease resistance, with greater accuracy. This enhances selection intensity and allows for greater genetic gain in a shorter timeframe, thereby increasing the overall efficiency of the breeding program.

3.4 Genomic Tools for Climate Resilience in Sugarcane

Sugarcane is a key crop used for the production of both sugar and bioethanol; however, climate change has made it increasingly difficult for farmers to maintain stable yields (Verma et al. 2022a, b). The development of genetically favorable cultivars that can survive—and even thrive under climate-altered conditions, such as irregular rainfall, rising temperatures, and more frequent and intense extreme weather events—is therefore essential (Devi and Kumar 2023a, b; Kumar and Kumar 2023; Sharma et al. 2023a; Sinam et al. 2023). Molecular tools have become indispensable in modern plant breeding, as they enhance both the precision and efficiency of the selection process, while also deepening our understanding of the genetic basis of climate-resilience traits. Genomic technologies enable the identification of specific genes and alleles linked to adaptive traits, which are critical under climate stress. This chapter will explore genomic approaches such as genome-wide association studies (GWAS), quantitative trait loci (QTL) mapping, and genomic selection (GS), highlighting their role in accelerating breeding programs and developing climate-resilient sugarcane cultivars.

3.4.1 Application of Genomic Tools for Identifying Climate Resilience Traits

3.4.1.1 Quantitative Trait Loci (QTL) Mapping

Quantitative trait loci (QTL) mapping is another widely used genomic tool in plant breeding, primarily employed to identify genomic regions associated with complex quantitative traits. These traits, such as heat stress tolerance, drought resistance, and disease resistance, are typically controlled by multiple genes. In QTL mapping, two parental lines are crossed, where one parent carries the desired trait (e.g., drought resistance). The resulting progeny—known as a segregating population—is then evaluated for both phenotypic traits (such as drought tolerance) and genotypic markers (such as SSRs, SNPs, or AFLPs). By comparing phenotypic data with molecular marker profiles, breeders can identify specific QTLs linked to the trait of interest, facilitating the selection of genotypes with favorable alleles through marker-assisted breeding (Karam et al. 2023; Aggarwal et al. 2024).

3.4.1.2 QTL Mapping for Climate Resilience Traits

QTL mapping has significantly contributed to the identification of genomic loci associated with climate adaptability in sugarcane and other crops. For instance, traits linked to drought resistance—such as root architecture, osmotic adjustment, and water use efficiency—have been successfully mapped using this approach.

Once these QTLs are identified, they can be used in further genetic studies or incorporated into commercial cultivars through marker-assisted selection (MAS). Similarly, QTL mapping has enabled the identification of genomic regions related to heat tolerance. These loci are associated with key physiological traits such as photosynthetic efficiency under high temperatures, leaf cooling mechanisms, and membrane stability (Dwivedi et al. 2020; Khew et al. 2022; Verma et al. 2022c, d; Wang et al. 2024; Zhang et al. 2024).

Because climate-resilience traits are complex and influenced by multiple environmental factors, identifying the underlying genetic regions through QTL mapping is essential. However, QTL mapping can be limited by low genetic variation between parental lines and insufficient segregating population sizes (Sharma and Kumar 2023). To overcome these challenges, breeders are increasingly turning to genome-wide association studies (GWAS), which offer greater resolution and access to broader genetic diversity, enhancing the identification of relevant adaptive traits.

3.4.1.3 Genome-Wide Association Studies (GWAS)

Genome-wide association studies (GWAS) represent a robust genomic approach for identifying phenotypic traits and genetic signatures within a large and diverse germplasm pool. Unlike QTL mapping, which relies on crosses between two distinct parental lines, GWAS utilizes genotypic data from naturally occurring populations, making it particularly effective in polymorphic populations. This approach allows for high-resolution gene mapping of complex, multifactorial traits, making GWAS a valuable tool in modern plant breeding programs.

3.4.1.4 GWAS for Climate Resilience Traits

When applied to sugarcane, genome-wide association studies (GWAS) have enabled the identification of genomic regions associated with key climate-adaptive traits such as drought tolerance, heat tolerance, and disease resistance. For example, the use of the GWAS platform—such as the Gorilla Whole-Genome Association Study—has led to the identification of SNPs linked to drought-related traits. These traits include physiological and morphological characteristics that enhance a plant's ability to conserve water, such as deep root systems, reduced leaf area, and delayed flowering until the onset of rainfall. Once SNPs associated with these features are identified, they can be developed into molecular markers for use in marker-assisted selection (MAS), thus accelerating the breeding of drought-tolerant cultivars. GWAS has also been used to explore traits linked to heat stress tolerance, such as photosynthetic efficiency, chlorophyll content, leaf heat conductance, and radiation use efficiency under high temperature. These traits are critical for maintaining productivity in sugarcane-growing regions that experience early-season heat stress.

Additionally, GWAS has been instrumental in identifying genes associated with disease resistance, especially as climate change intensifies pest and disease outbreaks under warm and humid conditions. One of the main advantages of GWAS is its ability to detect multiple genetic loci with small effects on complex traits, making it a powerful tool for dissecting the heritability of climate resilience traits. However, successful implementation of GWAS requires large, well-characterized populations with extensive phenotypic and genotypic data.

3.4.1.5 Marker-Assisted Selection (MAS)

Once QTLs or SNPs associated with climate resilience traits have been identified through QTL mapping or GWAS, breeders can use marker-assisted selection (MAS) to incorporate these traits into commercial cultivars. In MAS-based breeding programs, individual plants carrying the favorable allele at a specific locus—such as an SNP linked to drought tolerance—are selected for further breeding (Singh et al. 2015; Srinivasa Rao et al. 2016; Shahzad et al. 2022; Mangal et al. 2024). Molecular markers used in MAS allow breeders to select desirable traits at early developmental stages, thereby eliminating the need for time-consuming and costly phenotypic evaluations under field conditions.

3.4.1.6 MAS for Climate-Resilient Sugarcane

Marker-assisted selection (MAS) breeding programs in sugarcane have led to the development of favorable traits such as drought tolerance, disease resistance, and enhanced water use efficiency. For example, to accelerate the development of drought-tolerant varieties, breeders can use DNA markers linked to drought-related QTLs to identify individuals with the desired traits early in the breeding cycle. Similarly, markers associated with disease-resistance loci have been employed to select genotypes resistant to rust and sugarcane smut, particularly in warmer and more disease-prone regions. However, for complex traits such as drought and heat tolerance—which are polygenically controlled and influenced by multiple environmental factors—MAS is less effective than it is for simple traits governed by one or two major genes (also known as qualitative traits). To overcome this limitation, breeders are increasingly incorporating genomic selection (GS) as a complementary strategy, which allows for the prediction of breeding values based on genome-wide marker data and is more effective for improving complex, quantitative traits (Dubey et al. 2019; Ariyoshi et al. 2024).

3.4.2 Genomic Selection (GS) for Accelerating Breeding Progress

Genomic selection (GS) is a modern breeding approach that eliminates the need for extensive phenotypic evaluation by estimating an individual's breeding value based on genome-wide marker data. In GS, phenotypic and genotypic data from a reference population are used to train a statistical model, which can then predict the performance of other individuals using only their genotype information. Unlike marker-assisted selection, which targets specific loci, GS utilizes the entire genome, allowing it to capture the effects of both major and minor genes associated with complex polygenic traits, such as climate resilience (Henry 2020; Rai et al. 2020; Ahmar et al. 2023; Gantait et al. 2024; Hoffman et al. 2024).

3.4.2.1 Predicting Climate Resilience Traits with GS

The application of genomic selection (GS) offers immense potential to accelerate the development of sugarcane cultivars tolerant to climate change impacts. Compared to marker-assisted selection (MAS), GS is a more comprehensive approach, especially for complex traits such as heat stress tolerance, drought tolerance, and water use efficiency, which are influenced by multiple genes of varying effect sizes. By incorporating data from the entire genome and accounting for the combined influence of all genetic markers—including those with minor effects—GS minimizes prediction errors and enhances the accuracy of performance forecasts under specific environmental conditions. For example, traits such as root depth, leaf senescence, and osmotic adjustment have been used in GS models to predict drought tolerance in sugarcane (Wilson and VanBuren 2022; Reyes et al. 2023). Likewise, GS models trained on heat-tolerant populations can estimate yield potential in high-temperature environments. Genomic selection allows breeders to identify and select individuals with optimal genetic profiles for climate resilience before subjecting them to field phenotyping, thereby saving time and resources in breeding programs.

3.4.2.2 Advantages of GS for Breeding Climate-Resilient Sugarcane

One of the key advantages of genomic selection (GS) is its ability to reduce labor- and resource-intensive field trials, thereby accelerating the breeding process. GS allows for early selection of plants with climate-resilient traits by estimating their genetic potential rather than relying on phenotypic performance, which can significantly shorten the breeding cycle and enable quicker responses to emerging climate-related challenges. Another critical benefit of GS is its capacity for multi-trait selection, which is especially important for breeding climate-resilient varieties. GS enables the simultaneous selection of multiple traits—such as heat tolerance, drought resistance, and disease resistance—within a single population, increasing

the likelihood of developing genotypes that can perform well across diverse and challenging environments. This is particularly valuable for regions facing multiple stress factors concurrently, such as prolonged droughts combined with high temperatures or increased pest pressures exacerbated by warming climates.

3.4.2.3 Challenges and Future Prospects of GS

While genomic selection (GS) holds substantial promise, it is not without challenges—especially in polyploid crops like sugarcane, which possess multiple sets of chromosomes. The integration of all relevant genetic information into GS models is particularly complex in sugarcane due to its large genome size and widespread gene duplication. Furthermore, the effectiveness of GS is highly dependent on the quality of phenotypic data used for model training and the genetic diversity and reliability of the reference population. To address these limitations, researchers are focusing on strategies such as incorporating high-density genomic data, improving statistical methods for GS model development, and expanding the size and genetic diversity of reference populations. In addition, ongoing advancements in sequencing technologies and analytical software are expected to streamline the application of GS and reduce overall costs. The potential of GS can be further amplified when combined with other genomic tools like CRISPR-Cas gene editing, which enables precise modification of genes linked to climate resilience traits. By leveraging this combination, breeders can more accurately and efficiently identify and alter genes responsible for climate adaptation in sugarcane (Ahmar et al. 2023; Mangal et al. 2024; Patel et al. 2024).

Today, sugarcane breeders have access to a suite of powerful genomic tools—including GWAS, MAS, QTL mapping, and GS—that enhance the understanding of complex, multi-genic traits like drought tolerance, heat stress resistance, and disease resistance. Among these, genomic selection is considered the most promising for accelerating breeding progress. To foster climate resilience, breeders can now predict genotype performance under future climate scenarios and select individuals with greater adaptive potential. Incorporating genomic tools into breeding pipelines is essential for developing next-generation sugarcane cultivars suited to changing environmental conditions. Moreover, genetic engineering technologies offer long-term solutions for biofuel production and the sustainable cultivation of sugarcane, ensuring food and energy security for future generations (Dubey et al. 2019; Abdul Aziz and Masmoudi 2024; Gholizadeh et al. 2024).

3.5 Breeding Strategies for Climate Resilience

Developing climate-resilient sugarcane varieties is essential to ensure continued productivity amid emerging challenges such as heat stress, drought, pest outbreaks, and disease pressure. These climatic and biotic stresses affect sugarcane throughout

its growth cycle and demand innovative breeding strategies to preserve yield and crop quality. Sugarcane is a key commercial crop valued for its dual use in sugar and bioethanol production. To enhance its resilience under climate change, breeders employ a range of techniques, including conventional breeding methods, marker-assisted selection (MAS), and genetic engineering. Each of these approaches has its own set of advantages and limitations, making it important to assess their application based on specific breeding goals. This chapter explores both traditional and modern breeding strategies used to develop sugarcane cultivars with enhanced climate tolerance. These include hybridization, selection, MAS, and transgenic technologies aimed at introducing desirable traits. Furthermore, the strengths and weaknesses of these plant breeding approaches will be critically analyzed.

3.5.1 Conventional Breeding Methods: Hybridization and Selection

Over the years, two core components of traditional plant breeding—hybridization and selection—have been widely applied to develop improved crop varieties. In sugarcane breeding, crosses are made between genetically diverse parent lines, and offspring are selected based on phenotypic expression of desirable traits. Conventional breeding has played a critical role in enhancing climate-resilient traits in sugarcane, including heat tolerance, disease resistance, and drought tolerance.

3.5.1.1 Hybridization

The concept of hybridization in sugarcane focuses on creating new crop varieties that combine the favorable genes of two or more parental lines. For climate adaptation, breeders often cross drought-tolerant, heat-resistant, disease-resistant, or high-yielding cultivars to integrate multiple stress-resilient traits into a single genotype. Due to its high genetic variability, heterozygosity, and polyploidy, sugarcane is particularly well suited for hybridization, allowing the development of hybrids with enhanced genetic diversity and novel traits. To identify superior hybrids with both climate resilience and high productivity, breeders evaluate progenies across multiple generations and diverse environmental conditions. The primary objective is to select hybrids that consistently perform well under stress factors such as elevated temperatures and limited water availability.

3.5.1.2 Selection

The process of selecting offspring from a hybrid population that exhibit specific desirable traits is known as selection. This can be performed through phenotypic selection, where plants are chosen based on observable characteristics, or genotypic selection, where molecular tools are used to identify individuals carrying the desired

genes. Accurate selection requires robust germplasm characterization, often involving field experiments to assess traits such as heat tolerance, disease resistance, and drought tolerance. However, the selection process becomes increasingly complex for climate resilience traits, as these are typically controlled by multiple genes and influenced by environmental factors. For example, selecting for drought tolerance based solely on visible traits can be misleading, as underlying physiological factors—such as root architecture, water use efficiency, and osmotic adjustment—also play crucial roles.

3.5.2 Marker-Assisted Selection (MAS) for Targeted Introgression of Desirable Genes

Marker-assisted selection (MAS) refers to the use of molecular markers to identify and select individuals with desirable genetic traits for breeding. MAS enables breeders to target specific genes associated with favorable traits—such as drought tolerance, heat tolerance, or disease resistance—without relying solely on time-consuming and costly field trials. This approach has become a powerful and efficient tool in accelerating the development of climate-resilient sugarcane varieties, making it a cornerstone of modern breeding programs focused on adaptation to environmental stress.

3.5.2.1 How MAS Works

In marker-assisted selection (MAS), molecular markers such as single nucleotide polymorphisms (SNPs) and simple sequence repeats (SSRs) are used to identify genomic regions associated with desirable traits. For example, if a specific SNP is linked to drought tolerance, breeders can screen individuals for the favorable allele and select them for breeding. Because MAS targets genetic markers directly associated with traits of interest, it allows for more accurate selection than relying solely on phenotypic traits (Ahmad et al. 2020; Zhang et al. 2024). MAS is particularly effective for traits governed by simple genetic mechanisms, such as drought or heat tolerance, though it may be less effective for complex polygenic traits. A major advantage of MAS is the ability to make early selections—breeders can identify seedlings carrying the desired markers before exposing them to environmental stress. This reduces the need for extensive and costly field trials, thereby streamlining and accelerating the breeding process (Singh et al. 2015; Srinivasa Rao et al. 2016; Wang et al. 2024).

3.5.2.2 Application of MAS in Climate Resilience

Marker-assisted selection (MAS) has successfully contributed to the development of sugarcane cultivars with traits such as water-deficit tolerance, improved water use efficiency, and enhanced disease resistance. For instance, quantitative trait loci (QTLs) associated with drought tolerance have been identified in sugarcane, and molecular markers linked to these QTLs are now used to select individuals with improved drought resilience. Similarly, cultivars with superior traits like heat tolerance and disease resistance, especially in warm climates, are being integrated into breeding programs. MAS accelerates the breeding process by relying on genetic markers rather than extensive phenotypic evaluations. As a result, the need for field-based phenotype measurements is minimized, while the precision in selecting for complex traits is improved. This technique enables the development of high-yielding sugarcane varieties with genetic resilience to climate change and also facilitates the introgression of multiple traits within a single breeding cycle (Ahmad et al. 2020; Reyes et al. 2023). However, developing reliable molecular markers linked to target traits for MAS can be both time-consuming and costly. Therefore, MAS requires well-characterized germplasm and a comprehensive understanding of the genetic architecture of climate resilience traits. When it comes to polygenic traits governed by numerous genes with small to moderate effects, MAS is generally less effective than for traits controlled by major genes (Wilson and VanBuren 2022; Mangal et al. 2024).

Despite these limitations, MAS represents a significant advancement over traditional breeding methods, contributing to improved crop productivity under climate stress. Nevertheless, it does not fully address the complex challenges posed by climate change. As a result, breeders are increasingly turning to genetic engineering to enhance existing germplasm and develop new sugarcane cultivars with multiple desirable traits (Shahzad et al. 2022; Abdul Aziz and Masmoudi 2024; Ariyoshi et al. 2024; Gholizadeh et al. 2024).

3.5.3 Genetic Engineering for Introducing Novel Traits

Genetic engineering involves modifying an organism's genetic makeup by altering existing genes or introducing new genes directly into its DNA to control specific traits. From the perspective of climate resilience, genetic engineering is a transformative approach. It enables breeders to introduce traits that are not naturally present in the species—such as heat stress tolerance or enhanced water use efficiency—by incorporating genes from other organisms.

3.5.3.1 Techniques of Genetic Engineering

Another technique used in genetic engineering is transgenesis, which involves transferring genes from one organism into the genome of another to introduce new traits. A related approach is genetic modification using tools such as CRISPR/Cas9, which allows precise editing of a plant's own genes to enhance or suppress specific traits.

3.5.3.2 Transgenesis

Transgenic breeding is a technique applied in sugarcane to introduce beneficial traits by incorporating genes from other species. For example, to develop sugarcane varieties with improved drought tolerance, genes from drought-resistant species such as Arabidopsis or rice can be inserted into the sugarcane genome. These transgenes enhance the plant's ability to withstand water scarcity by regulating stress-response mechanisms.

3.5.3.3 CRISPR/Cas9 Gene Editing

It is now possible to selectively modify climate-resilient genes in sugarcane using CRISPR/Cas9, a tool that enables targeted editing of the sugarcane genome. For instance, CRISPR can be used to enhance beneficial regulatory or housekeeping genes associated with water use efficiency (WUE), or to disable genes that contribute to heat stress susceptibility. Genetically engineered sugarcane cultivars designed to tolerate climate stress offer several advantages. For example, to enhance drought tolerance, genes that regulate stomatal closure, promote root system development, or improve osmotic adjustment may be introduced. Similarly, sugarcane lines with enhanced photosynthetic efficiency and heat-stable proteins have been developed through genetic modification. Genetic engineering also allows the introduction of traits not naturally found within the sugarcane germplasm, which traditional breeding cannot achieve. A modern solution to combat emerging threats includes transferring pest- or disease-resistance genes from other plant species, potentially protecting sugarcane from pathogens thriving in warmer climates. In cases where conventional breeding and recurrent selection fail to resolve specific challenges, genetic engineering provides a precise method to introduce new traits. Compared to other genetic techniques, this method offers greater control over specific genetic alterations, minimizing unintended changes in the DNA (Kumar et al. 2013; Singh et al. 2021).

Additionally, the development of genetically engineered cultivars is significantly faster than traditional crossbreeding, which requires multiple generational cycles (Kumar et al. 2021, 2022b; Pathak et al. 2017). To ensure sustainable sugarcane

cultivation in the face of climate change, farmers must adopt climate-resilient cultivars (Verma et al. 2022e). Although selection and hybridization have been used to improve genetic diversity and introduce adaptive traits, these approaches are time-consuming and labor-intensive. Marker-assisted selection (MAS) has improved breeding efficiency by allowing trait selection based on molecular markers. Genetic engineering further expands breeding capabilities by enabling gene transfer from unrelated species, beyond the conventional gene pool. Given regional variations in climate challenges, breeders must choose context-specific strategies (Aley et al. 2021; Das et al. 2022). Each approach—whether conventional breeding, MAS, or genetic engineering—has distinct strengths and limitations. Ultimately, a combination of traditional methods with advanced biotechnology and MAS offers the most effective solution for developing sugarcane varieties adapted to increasingly stressful environments caused by climate change (Kumar et al. 2011; Chakraborty et al. 2021; Goud et al. 2022; Saini et al. 2023).

3.6 Case Studies of Climate-Resilient Sugarcane Varieties

These factors include drought, heat stress, salinity, and the emergence of new pests and diseases, which pose significant threats to sugarcane growth under the influence of global warming. Regions that rely on sugarcane for food and biofuel must prioritize the development of improved, climate-resilient sugarcane varieties to safeguard sugarcane cultivation and its associated industries. In response to these challenges, breeding initiatives have been undertaken globally to develop sugarcane cultivars capable of withstanding adverse environmental conditions. This chapter highlights breeding programs that have successfully produced sugarcane varieties resilient to climate change. It will also evaluate the traits and performance of these varieties under different stress conditions and demonstrate their role in improving sugarcane sustainability and productivity. As climate change becomes increasingly unpredictable, the development of durable climate-resilient sugarcane cultivars is essential for long-term sustainability. Countries such as the USA, South Africa, Brazil, Thailand, and India have made notable progress in breeding sugarcane varieties resistant to abiotic stresses such as heat, drought, flooding, and biotic stresses like pests and diseases. Beyond yield enhancement and increased farmer profitability, the adoption of these resilient cultivars has contributed to reduced reliance on chemical inputs and water, thereby promoting sustainable farming practices.

To maintain stability in sugarcane-based economies under evolving climate risks, it is imperative to continue developing and adopting new and improved sugarcane varieties. These efforts are crucial as climate change introduces unprecedented challenges to global agriculture (Guo et al. 2023).

3.6.1 The Co 0238 Sugarcane Variety in India

A significant portion of India's sugar and ethanol production is dependent on sugarcane, and India has developed several noteworthy climate-resilient varieties. One prominent example is the Co 0238 variety, developed by the Sugarcane Breeding Institute (SBI) in Coimbatore, Tamil Nadu. This variety revolutionized sugarcane cultivation in India due to its exceptional adaptability and performance under climatic stress. Co 0238 was selectively bred to combine high yield potential with strong tolerance to drought and other environmental adversities, making it a cornerstone for sustainable sugarcane farming in the country.

3.6.1.1 Traits and Performance

Some distinctive characteristics of Co 0238 include high sucrose content, early maturity, and consistently high yield under both normal and stress conditions. It also exhibits superior water-use efficiency compared to conventional varieties. The deep-penetrating root system of Co 0238 enables it to access moisture from deeper soil layers, making it highly drought-resistant. Field trials and observations have shown that while many sugarcane varieties are susceptible to red rot and smut, Co 0238 has demonstrated strong resistance to both diseases. Additionally, its resistance to pest attacks makes it a preferred choice for seed cane, especially in regions prone to disease outbreaks, where farmers highly value its resilience and stability.

3.6.1.2 Impact on Production and Sustainability

Sugarcane production in India has experienced a significant boost with the introduction of the Co 0238 variety. This cultivar has delivered exceptionally high yields to farmers, primarily due to its enhanced cane productivity and improved sugar recovery rates. One of the highest recorded sugar recovery rates in India—11.5%—was achieved in Uttar Pradesh, the country's largest sugarcane-producing state, following the widespread adoption of Co 0238. This variety has greatly contributed to the region's agricultural sustainability, as it maintains consistent yields even under erratic or low rainfall conditions. Thanks to the superior yield potential and stress resilience of Co 0238, climate-related challenges have had minimal impact on farmers' ability to sustain optimum production levels. Consequently, this has supported food security, improved rural livelihoods, and had a positive impact on the national economy (Choudhury et al. 2024; Devi and Kumar 2024; Pandey et al. 2024; Dey et al. 2024).

3.6.2 The RB867515 Variety in Brazil

As the world's leading producer of sugarcane and bioethanol, Brazil has taken a pioneering role in developing sugarcane cultivars resilient to adverse climatic conditions. One of the most successful varieties currently cultivated in Brazil is RB867515, developed by RIDESA (the Inter-University Network for the Development of the Sugarcane Industry). This variety was bred with two major goals in mind: enhancing ethanol yield and improving drought tolerance.

3.6.2.1 Traits and Performance

Due to the impacts of climate change, RB867515 has gained increasing importance in Brazil's sugarcane-producing regions because of its remarkable drought tolerance. Its ability to maintain consistent biomass production during dry periods makes it highly resilient to drought. Even under erratic rainfall conditions, RB867515 continues to grow and maintain productivity. In addition to its drought resistance, the variety exhibits strong resistance to major sugarcane diseases, including orange rust and leaf scald. RB867515 has demonstrated high yields under Brazilian field conditions, showing robust tolerance to both biotic and abiotic stresses, making it a reliable choice for farmers confronting climate variability.

3.6.2.2 Impact on Production and Sustainability

Sugarcane cultivation in Brazil has significantly benefited from the adoption of RB867515, which has led to enhanced yields and improved economic returns. In major production zones such as São Paulo State—Brazil's leading sugarcane-producing region—RB867515 now accounts for a substantial portion of the total area under cultivation, making it one of the most widely grown sugarcane varieties in the country. This variety has been particularly valuable for Brazil's ethanol industry due to its high productivity and extended ratoon longevity. The ethanol sector is essential not only for domestic energy needs but also for export markets. RB867515 has enabled the expansion of sugarcane cultivation into areas with limited water availability, thus reducing dependence on water-intensive farming. Its adaptability has helped farmers manage climate-related challenges and contributed to the sustainability of agricultural practices in Brazil.

3.6.3 The KPS 94-10 Variety in Thailand

Sugarcane is a vital agricultural commodity for the Thai economy, and Thailand holds a prominent position among Southeast Asian (SEA) sugarcane-producing countries. However, the increasing instability of the climate—marked by more

frequent droughts and fluctuating temperatures—poses a serious challenge to sugarcane cultivation in the region. To address this issue, Kasetsart University, in collaboration with the Thai government, developed a climate-resilient sugarcane cultivar known as KPS 94-10.

3.6.3.1 Traits and Performance

KPS 94-10 is recognized globally for its drought resistance and high performance, even under adverse growing conditions. This cultivar was specifically selected for its robust root architecture, which enhances the plant's ability to absorb water, thereby reducing its vulnerability to drought stress. Additionally, KPS 94-10 exhibits excellent heat tolerance, making it well suited for regions with high summer temperatures. The sugarcane borer, a major pest in Thailand—particularly in tropical zones—poses a serious threat to sugarcane crops. However, KPS 94-10 demonstrates strong resistance to insect pests and other common pathogens, significantly reducing the need for chemical pesticide applications. This insect resistance enhances the cultivar's ecological value by promoting more sustainable and environmentally friendly sugarcane production.

3.6.3.2 Impact on Production and Sustainability

It can be stated that KPS 94-10 has had a significant impact on the Thai sugarcane industry. Despite increasingly erratic climatic conditions, this cultivar has enabled stable sugarcane production under both drought and extreme heat. This has benefited not only the local market but also export-oriented sugar companies by helping stabilize Thailand's overall sugar output. Furthermore, the variety's inherent resistance to pests has led to a reduction in pesticide usage, which is advantageous for environmental sustainability, economic viability, and overall farming practices. By cultivating KPS 94-10, farmers are able to minimize their reliance on toxic agrochemicals, thus promoting safer and more eco-friendly agricultural systems.

3.6.4 The CP 96-1252 Variety in the United States

Currently, the majority of sugarcane in the United States is cultivated in states like Florida and Louisiana, regions increasingly vulnerable to climate change-related challenges such as storms and flooding. To address these threats, the USDA's Agricultural Research Service (ARS), in collaboration with the University of Florida, developed the sugarcane variety CP 96-1252. This cultivar serves as a prime example of a climate-resilient type, playing a significant role in mitigating the adverse impacts of climate change on sugarcane production.

3.6.4.1 Traits and Performance

CP 96-1252 is well known for its high resistance to flooding and waterlogging—two major challenges now emerging in sugarcane-growing regions due to rising sea levels and increasingly severe storms. Since standing water can be detrimental to traditional sugarcane varieties, CP 96-1252 was specifically developed to withstand such adverse conditions. In addition to its waterlogging tolerance, CP 96-1252 also exhibits strong resistance to several diseases, particularly those that thrive in wet and humid climates, such as mosaic virus and leaf scald. Because of these characteristics, CP 96-1252 is especially well suited for the Gulf Coast region, where climate change poses a serious threat to sugarcane cultivation.

3.6.4.2 Impact on Production and Sustainability

CP 96-1252 has played a vital role in maintaining sugarcane yields in flood-prone regions. Its resilience to prolonged waterlogging has helped prevent crop failures in the face of extended flooding, offering stability amid increasingly unpredictable weather conditions. As a result, sugarcane production has improved, ensuring consistent profitability for growers in climate-vulnerable states such as Louisiana and Florida. Furthermore, the strong disease resistance of CP 96-1252 reduces the reliance on fungicides and other agrochemicals, thereby supporting more environmentally friendly agricultural practices. This cultivar's resilience not only safeguards yields but also promotes sustainable farming by lowering farmers' dependency on chemical inputs (Singh et al. 2015; Shahzad et al. 2022; Ahmar et al. 2023; Sharma et al. 2023a; Ariyoshi et al. 2024; Kumar and Singh 2024b).

3.6.5 The NCo376 Variety in South Africa

South Africa is another major sugarcane-producing country, where farmers frequently face extreme climate stressors such as heat and drought. To address these challenges, the South African Sugarcane Research Institute (SASRI) developed the variety NCo376, a climate-resilient cultivar that has gained widespread acceptance across the country.

3.6.5.1 Traits and Performance

NCo 376 is widely recognized for its ability to perform well under drought conditions and maintain high productivity in arid climates. This resilience is attributed to a combination of traits, including a highly efficient root system that extracts water

from deeper soil layers during dry spells, enabling the variety to withstand prolonged drought. Additionally, NCo 376 demonstrates strong heat tolerance, maintaining active photosynthesis and biomass accumulation even under extreme temperatures. The variety also exhibits moderate resistance to rust and smut—two significant diseases that become more problematic in regions experiencing harsh climatic conditions. Its combined resistance to both biotic and abiotic stressors has made it a preferred choice among South African farmers.

3.6.5.2 Impact on Production and Sustainability

As previously noted, sugarcane production in South Africa has significantly benefited from the adoption of the NCo376 cultivar, particularly in drought-prone regions. This variety has played a vital role in stabilizing the nation's sugarcane industry—an essential component of the agricultural economy—by ensuring consistent yields under water-limited conditions. Furthermore, the drought tolerance of NCo376 has led to more efficient utilization of scarce water resources, a critical factor in regions with limited rainfall such as parts of South Africa. In addition, its resistance to major diseases has reduced the need for frequent chemical applications, promoting environmentally friendly agricultural practices. The South African case demonstrates how targeted breeding efforts to improve sugarcane resilience to climate stress have made cultivars like NCo376 indispensable.

3.7 Challenges and Future Prospects in Breeding Climate-Resilient Sugarcane Varieties

Breeding new sugarcane varieties that enhance productivity and adapt to diverse climate change challenges is becoming increasingly important due to global environmental shifts. Sugarcane remains one of the most essential crops for the production of sugar and bioethanol, yet it is highly sensitive to environmental stresses such as drought, high temperatures, salinity, pests, and diseases. Despite advancements in breeding techniques, breeders continue to face significant obstacles in developing sugarcane cultivars that are resilient to these stressors. This chapter aims to examine these challenges, evaluate the future potential of germplasm utilization and breeding technologies, and emphasize the importance of collaborative efforts in addressing climate change impacts on sugarcane agriculture.

3.7.1 Challenges in Breeding Climate-Resilient Sugarcane Varieties

3.7.1.1 Complex Genetic Structure and Long Breeding Cycles

One of the major challenges for scientists is the genetic complexity of the sugarcane genome, which hinders the rapid development of climate-resilient sugarcane varieties. Sugarcane is a polyploid crop, possessing between 80 and 120 chromosomes—significantly more than most other crops. This complex genetic architecture makes conventional breeding methods difficult and time-consuming, as breeders must select desirable traits from an enormous pool of genetic possibilities. Additionally, sugarcane is propagated vegetatively, which further complicates and prolongs the breeding process. Developing a new climate-resilient sugarcane variety typically requires 10–15 years, during which the environmental conditions under which the variety was initially selected may change. As a result, by the time the new variety is ready for release, it may already be less suited to current climatic realities. This slow pace of breeding progress significantly limits the ability to adapt sugarcane production to rapidly evolving climate conditions (Srinivasa Rao et al. 2016; Singh et al. 2015; Reddy et al. 2022; Kumari et al. 2022; Kumar et al. 2021; Goud et al. 2022; Gantait et al. 2024; Dwivedi et al. 2020; Ariyoshi et al. 2024).

3.7.1.2 Drought, Heat, and Salinity Tolerance

Water scarcity and high-temperature stress are among the most critical challenges facing sugarcane production, particularly in tropical and subtropical regions. Selecting traits such as a deep root system, efficient water use, or heat-tolerant photosynthesis for breeding drought- and heat-resilient varieties is difficult, primarily because these traits are controlled by multiple genes, making their identification and incorporation into new cultivars complex. Furthermore, there is often a trade-off between stress tolerance (drought and heat) and yield potential, making it especially challenging to develop cultivars that are both high-yielding and resilient under stress conditions. Salinity stress is another major concern in sugarcane-growing regions, particularly in coastal areas affected by rising sea levels or where irrigation water is insufficient or of poor quality. High salt concentrations can inhibit plant growth and significantly reduce sugar yield. To breed new sugarcane varieties capable of maintaining or improving yields under saline conditions, extensive screening of diverse germplasm collections is required to identify salt-tolerant genes—an effort that is both laborious and time-intensive.

3.7.1.3 Disease and Pest Resistance

Climate change is introducing new pests and diseases into regions where they were previously unknown, posing a growing threat to sugarcane cultivation. Sugarcane is particularly vulnerable to pests such as the black sugarcane borer, and diseases like smut, mosaic virus, and red rot. Developing sugarcane varieties that are resistant to such biotic stresses is essential; however, this is often challenging because pests and pathogens can rapidly evolve to overcome resistance mechanisms. Moreover, introducing resistance genes into commercial sugarcane varieties is complicated by the crop's polyploid nature, which involves multiple sets of chromosomes (Rai et al. 2020; Gantait et al. 2024). Additionally, biotic stress resistance is often location-specific, meaning that cultivars bred for resistance in one region may not be equally effective in another. This makes it difficult for breeders to develop universally resistant sugarcane varieties, especially in the face of the unpredictable emergence and spread of pests and diseases driven by climate change (Srinivasa Rao et al. 2016; Henry 2020; Abdul Aziz and Masmoudi 2024; Ariyoshi et al. 2024).

3.7.1.4 Limited Germplasm Diversity

A lack of genetic variation remains one of the most critical challenges in developing climate-resilient sugarcane varieties. Most modern sugarcane cultivars are derived from a narrow genetic base, primarily involving a few Saccharum species. As a result, limited genetic diversity constrains the development of novel traits, such as heat tolerance, drought resilience, and pest resistance. Although, as noted earlier, wild relatives of sugarcane offer valuable untapped genetic resources, incorporating their traits into cultivated varieties is laborious and time-consuming, due to cross-incompatibility and limited reproductive compatibility. Additionally, sugarcane germplasm collections are often inadequately conserved or poorly shared, especially in some sugarcane-growing regions or countries. This further limits breeders' access to a diverse range of parental germplasm, which is essential for developing cultivars capable of adapting to changing climatic conditions.

3.7.2 Future Prospects for Germplasm Utilization and Breeding Approaches

Nevertheless, these challenges have paved the way for new opportunities to advance the development of climate-resilient sugarcane cultivars. Key opportunities to address the limitations in sugarcane breeding include the integration of advanced genomic resources, high-throughput phenotyping technologies, and multi-institutional collaborative efforts (Table 3.1).

Table 3.1 A detailed table discussing sugarcane germplasm in terms of description, types, benefits, and disadvantages

Aspect	Description	Types	Benefits	Disadvantages	Examples
Germplasm	Genetic material for breeding programs contains hereditary information for traits like yield, disease resistance, etc.	Wild relatives, cultivated varieties, and hybrids	Source of diverse traits like stress resistance, higher yield, and disease tolerance	May lack specific characteristics, making it challenging to identify valuable genes	*S. officinarum* (cultivated), *S. spontaneum* (wild)
Wild relatives	Non-domesticated species related to sugarcane that offer beneficial traits for stress tolerance, disease resistance, etc.	*S. spontaneum, S. robustum*	Greater environmental stress tolerance, unique genetic traits	Difficulties in crossbreeding with cultivated varieties due to genetic barriers	*S. spontaneum* for drought tolerance
Cultivated varieties	Sugarcane varieties are bred for high yield and sugar content and are typically used in commercial farming	Modern hybrids, regional varieties	High productivity, well adapted to optimal conditions	Limited genetic diversity makes them vulnerable to climate and pest pressures	Co 0238 (high-yielding commercial variety)
Landraces	Traditional sugarcane varieties that are locally adapted are often used by small-scale farmers	Regional/local varieties	Well suited to specific environments, provides unique traits	Lower yield potential compared to modern varieties	Traditional varieties from India or Brazil
Hybrid varieties	Crossbreeds of different sugarcane species combine beneficial traits like disease resistance and high yield	*Saccharum* hybrids	Combines desirable traits from multiple sources, increases productivity	Can reduce genetic diversity if too reliant on hybrids	Co 86032 (high-yield, stress-tolerant hybrid variety)
In situ conservation	Conservation of germplasm in its natural habitat, where it continues to evolve with the environment	Natural reserves, protected areas	Allows for ongoing natural evolution and adaptation	Long-term survival of particular species may not be guaranteed due to habitat loss	Wild *Saccharum* spp. Conserved in biodiversity reserves
Ex situ conservation	Germplasm storage outside its natural environment is typically done in gene or seed banks	Gene banks, seed banks, cryopreservation	Ensures long-term preservation of genetic material for future breeding	It is expensive to maintain and requires continuous management	Sugarcane gene banks at ICAR-SBI, India
Primary germplasm	Germplasm from the main sugarcane species used in breeding programs	*Saccharum* spp.	Rich source of critical traits like high sugar content and yield	Limited traits related to stress or disease resistance	*S. officinarum* for high sugar content

(continued)

Table 3.1 (continued)

Aspect	Description	Types	Benefits	Disadvantages	Examples
Secondary germplasm	Germplasm from species closely related to sugarcane but less commonly used in breeding	*Miscanthus, Erianthus*	Introduces unique traits like cold tolerance and pest resistance	It is challenging to hybridize due to genetic distance	Miscanthus for cold tolerance traits
Tertiary germplasm	Genetic material from distantly related species often requires advanced techniques for use in breeding programs	Distant relatives, wild grasses	Source of rare traits that are unavailable in close relatives	Requires advanced technologies like genetic engineering for effective utilization	Distant grass relatives are used for improving biomass production
Phenotypic diversity	Variation in visible traits like stalk height, thickness, and disease resistance within sugarcane germplasm	Morphological traits, agronomic traits	Easily observable and selectable traits for breeding	Environmental factors can affect expression, complicating trait evaluation	Selection of sugarcane varieties with increased drought resistance
Molecular diversity	Genetic variation at the DNA level is essential for identifying specific genes associated with desirable traits	DNA markers, genomic tools	Enables precise identification of traits, facilitating targeted breeding	Requires advanced tools and expertise, and is time-consuming to analyze	Use of SSR markers for identifying disease resistance genes
Drought tolerance	The ability of sugarcane varieties to survive and maintain productivity under water stress	Drought-tolerant varieties	Improves sugarcane's ability to thrive in regions with water scarcity	Breeding for drought tolerance can take several generations	*S. spontaneum* hybrids bred for drought tolerance
Disease resistance	The capacity of certain sugarcane varieties or germplasm to withstand diseases like red rot, smut, and mosaic virus	Resistant varieties, wild relatives	Reduces the impact of diseases on sugarcane yield, enhancing farm productivity	Resistance may not be permanent as pathogens evolve	Co 89003 bred for resistance to red rot
Marker-assisted selection	The use of molecular markers to assist in selecting traits during breeding programs	DNA markers, molecular markers	Speeds up the breeding process by selecting traits at early growth stages	High cost of implementation and requires technical expertise	MAS is used to select for traits like disease resistance and sugar content
Pre-breeding	Preparing wild germplasm involves crossing it with cultivated varieties to introduce new traits	Wild relatives, interspecies hybrids	Introduces novel traits that are not available in existing cultivated varieties	Time-consuming, requires significant resources for testing and evaluation	Pre-breeding using *S. spontaneum* to improve stress tolerance

Genetic engineering	Directly manipulating sugarcane's genome to introduce desirable traits like stress tolerance or higher yield	CRISPR, transgenic technology	Introduces traits that are difficult to achieve through traditional breeding	Regulatory hurdles, ethical concerns, and potential environmental risks	Genetically modified sugarcane with increased herbicide tolerance
Collaborative research	Partnerships between research institutions, governments, and private companies to share germplasm and breeding knowledge	International consortia, public-private partnerships	Facilitates the sharing of diverse germplasm, resources, and technologies	Intellectual property rights and access agreements can complicate collaboration	Global Sugarcane Research Network, International Sugarcane Biotechnology Consortium (ISBC)
Germplasm evaluation	Assessment of germplasm for traits like yield, disease resistance, and stress tolerance through field trials or molecular analysis	Field trials, molecular marker analysis	Identifies the best-performing germplasm for breeding programs	Time-consuming and resource-intensive	Field evaluations of sugarcane varieties for performance under drought conditions
Gene banks	Facilities for storing and preserving sugarcane germplasm for long-term use in breeding and research	Seed banks, cryopreservation, and field collections	Long-term preservation of genetic diversity	Expensive to maintain and manage effectively	CIRAD gene bank (France), ICG (India)

3.7.2.1 Expanding Germplasm Utilization

Expanding sugarcane germplasm collections, including wild relatives and related species, is one of the most effective strategies to overcome the challenge of limited genetic diversity in sugarcane. Wild relatives often possess traits that confer greater tolerance to environmental stresses such as heat, drought, and disease, making them valuable resources for breeding. Modern techniques such as pre-breeding and genetic introgression can facilitate the incorporation of beneficial traits from wild relatives into commercial sugarcane cultivars. To strengthen the genetic base, it is essential to broaden germplasm collections and ensure open access to breeders worldwide. In addition to international cooperation for germplasm conservation, innovations in cryopreservation and in vitro conservation technologies offer promising approaches for preserving valuable genetic material. These techniques enable the long-term storage and effective utilization of germplasm, ensuring it remains available for future breeding initiatives.

3.7.2.2 Genomic Tools for Precision Breeding

The recent advancement of genomic technologies, such as genome sequencing, genome-wide association studies (GWAS), and quantitative trait loci (QTL) mapping, has brought about a transformative shift in sugarcane breeding strategies. These technologies enable breeders to identify specific genes and genetic markers linked to climate-related traits such as disease resistance, heat tolerance, and drought resilience. The integration of genotypic and phenotypic data supports more precise selection of parental lines, facilitating the development of improved varieties within a shorter time frame. Techniques like genomic selection (GS) and marker-assisted selection (MAS) are particularly valuable in accelerating the breeding process. MAS allows for the targeted introgression of desirable traits into new sugarcane populations, while GS predicts a plant's performance based on its genetic profile, enabling the early identification of superior genotypes. By using these genomic tools, breeders can reduce the reliance on extensive phenotypic evaluations, shorten breeding cycles, and improve the accuracy of selecting climate-resilient traits. These innovations are increasingly critical as climate change intensifies, necessitating rapid and efficient breeding responses.

3.7.2.3 Genetic Engineering and CRISPR Technologies

Another promising approach to developing sugarcane varieties capable of withstanding the impacts of climate change is genetic modification. Through genetic engineering, breeders can introduce novel traits that are not naturally present in the sugarcane gene pool. For instance, genes from other crops or wild relatives can be transferred into sugarcane to enhance resistance to heat, drought, or pests. Genetically modified (GM) sugarcane cultivars have already been developed with

enhanced pest and disease tolerance. However, public acceptance of GM crops and stringent regulatory frameworks remain significant challenges. A more recent and precise genetic tool is CRISPR/Cas9 gene editing, which offers a targeted and efficient method for improving sugarcane traits related to climate resilience. CRISPR allows breeders to precisely edit specific genes associated with stress tolerance without introducing foreign DNA, thereby potentially addressing some of the regulatory and consumer concerns linked with conventional GM crops. Although sugarcane breeding using CRISPR is still in its early stages, the technology holds immense potential for developing climate-resilient sugarcane varieties in the near future.

3.7.3 Need for Collaborative Research and Development Efforts

The analysis highlights that climate change is creating multiple challenges that must be addressed by researchers, policymakers, and industry stakeholders to ensure the long-term sustainability of sugarcane breeding programs. Global collaboration is essential for sharing knowledge, resources, and advanced technologies, which are critical for developing sugarcane varieties capable of withstanding the adverse impacts of climate change.

3.7.3.1 International Collaboration

Collaboration among institutions and countries is crucial for pooling resources and expertise in sugarcane development. International cooperation has advanced significantly through platforms such as the Global Sugarcane Network and the International Sugarcane Biotechnology Consortium (ISBC). These initiatives facilitate the exchange of germplasm, support the adoption of advanced breeding technologies, and fund collaborative research focused on climate change adaptation. Furthermore, bridging the gap between developed and developing sugarcane-producing countries involves understanding region-specific challenges and implementing targeted solutions. International partnerships enable nations with limited research infrastructure to access cutting-edge tools and knowledge, while breeding programs in drought-prone regions can benefit from research outcomes in countries with similar environmental conditions.

3.7.3.2 Public-Private Partnerships

Furthermore, the involvement of private companies and public-private partnerships (PPPs) is crucial for advancing sugarcane breeding programs. Many key developments, such as the creation of new breeding platforms and the application of genomic

tools, have emerged through collaboration among universities, private sector entities, and government institutions. These partnerships help bridge the gap between research and practice, enabling farmers to effectively implement and commercialize climate-resilient cultivars in a timely manner. Another significant advantage of PPPs is their ability to mobilize the financial and material resources required to support long-term breeding initiatives. Given that sugarcane breeding is both cost-intensive and time-consuming, a sustained financial commitment is essential for the continuous development of new varieties. Public-private partnerships play a pivotal role in securing consistent funding necessary to maintain and expand breeding programs.

3.7.3.3 Capacity Building and Knowledge Transfer

Securing and sharing knowledge is equally essential alongside cooperative research activities to ensure that all sugarcane-growing regions have the capacity and expertise required to develop climate-resilient cultivars. This involves equipping researchers and breeders with advanced tools such as gene editing technologies, genomic selection platforms, and training in modern breeding methodologies. Since sugarcane is a key source of income and employment in many developing countries, capacity building becomes a critical priority. However, these regions often lack the infrastructure and funding to support cutting-edge breeding research, making them more vulnerable to the impacts of climate change. International organizations, governments, and private sector entities must work collaboratively to provide financial and technical support, thereby enabling these regions to adapt their sugarcane production systems to evolving environmental challenges.

There is no doubt that the continuous breeding of climate-resilient sugarcane cultivars is a complex and demanding task, yet it is fundamental to ensuring the global sustainability of sugarcane production. Challenges such as the genetic complexity of sugarcane, long breeding cycles, and the pressing need to improve traits like heat tolerance, drought resilience, and disease resistance continue to hinder rapid progress. However, the application of germplasm resources, molecular genetics, and genomic tools offers promising pathways to develop climate-smart sugarcane varieties. To realize this potential, researchers, governments, and industry stakeholders must deepen their collaboration to support innovation and implementation. Global partnerships, private sector involvement, and focused capacity development initiatives will be vital to secure the long-term viability of sugarcane production and to effectively confront the challenges posed by climate change.

3.8 Conclusion and Future Research Directions

Another critical approach to increasing output is the strategic utilization of germplasm to develop new sugarcane varieties that are more resilient to the effects of climate change. Identifying traits such as drought tolerance, heat resistance, and

disease resistance within the germplasm of wild relatives and landraces provides a valuable resource for breeding. Genetic diversification empowers breeders to develop novel cultivars that are better equipped to withstand environmental stressors and climatic variability. A major breakthrough that has enhanced breeding efficiency and enabled the identification of climate-resilient traits is the adoption of modern breeding tools such as genomic selection (GS), CRISPR gene editing, genome-wide association studies (GWAS), and marker-assisted selection (MAS). To effectively address challenges posed by climate variability, it is essential to focus on the collection, conservation, and targeted utilization of germplasm, while applying advanced breeding technologies. Particular attention must be given to abiotic stressors such as drought and salinity, as well as emerging pests and diseases, to sustain high productivity and maintain the profitability of sugarcane cultivation.

By integrating phenotypic and genotypic data, breeders can unlock new opportunities for precision breeding, selectively enhancing sugarcane's resilience to climate stress. Moving forward, researchers must explore effective strategies for preserving germplasm, increasing accessibility, and integrating cutting-edge genomic insights into breeding pipelines. Recent technological advancements in gene editing and genetic engineering offer significant potential for developing sugarcane varieties with tailored traits for climate adaptation. The global exchange of genetic materials and resources for breeding will require strong national and international research collaboration. To secure the future of sugarcane production, it is essential to develop climate-resilient cultivars through the strategic use of diverse germplasm. Investing in the protection of sugarcane against climate change is a long-term commitment to safeguarding a vital crop that supports stakeholders from farmers to consumers. As a primary commodity, its sustainability hinges on continued research funding and cooperative technological innovation.

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Chapter 4
Microsatellites for Analysis of Genetic Diversity and Relationships in Different Sugarcane Varieties: An Overview

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Abstract Sugarcane from diverse ecological regions exhibits significant genetic diversity and is considered a rich source of novel or unique alleles. Microsatellites, also referred to as simple sequence repeats (SSRs), are DNA markers characterized by brief, repetitive motifs with varying repeat numbers at specific loci. These highly reproducible markers are crucial tools for genetic analysis. The genetic diversity of germplasm resources plays a vital role in crop improvement programs, as it determines the potential for sustained genetic advancements. SSR fingerprinting is widely employed in genetic diversity studies due to its high level of polymorphism. It allows for identifying allelic variations even among closely related varieties, enabling accurate assessment of genetic diversity in different sugarcane varieties. SSR analysis provides valuable insights into the unique genetic patterns and relationships between different varieties by targeting specific regions of the sugarcane genome. The molecular data generated from the analysis offers crucial guidance in various aspects of sugarcane breeding. It aids in selecting elite parent genotypes for crossing, establishing effective cross-combinations, designing hybrid breeding strategies, and helping address the challenges posed by climate change. Furthermore, the diverse sugarcane panel holds potential for further exploration in genome-wide association studies (GWASs), opening up new avenues for research and crop improvement.

Keywords Simple Sequence Repeats (SSRs) · Motifs · Climate · GWAS

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4.1 Introduction

Sugarcane (*Saccharum* spp.) is one of the world's most significant and versatile crops, predominantly grown in tropical and subtropical regions, essential to both the agricultural sector and the global economy. More than 80% of the world's sugar and 40% of its bioethanol output come from sugarcane, the world's top sugar producer and a promising source for bioenergy production (Bao et al. 2024; Verma et al. 2024a, b). Due to its many effects on nutrition, the environment, society, and economy, sugarcane cultivation is an essential endeavor on a global scale (Verma et al. 2021; Huang et al. 2024). Because of its economic and ecological advantages, nations like Brazil produce more sugarcane than any other country in the world. Furthermore, sugarcane bagasse, a lignocellulosic substance, is used as feedstock and biofuel in the paper industry, among other purposes (Sajid et al. 2023; Afghan et al. 2024). To produce an improved sugarcane variety, genetic diversity must exist. Higher biomass and/or sucrose production rates may result from better management of genetic resources found in germplasm banks and core collections (Medeiros et al. 2020; Verma et al. 2024c). Traditional sugarcane breeding methods are time-consuming and somewhat unsuccessful due to the crop's large genome size and high heterozygosity (Liang et al. 2024). Transgenic breeding must be used in breeding programs to expand the genetic basis or gene pool.

The genetic diversity across extensive germplasm collections of several *Saccharum* spp., modern sugarcane cultivars, and related progenitor species has been assessed in recent years using molecular markers (Singh et al. 2011). These molecular markers include sequence-related amplified polymorphism (SRAP), target region amplification polymorphism (TRAP), cleaved inter-simple sequence repeat (CISP), restriction fragment length polymorphism (RFLP), random amplified polymorphic DNA (RAPD), amplified fragment length polymorphism (AFLP), inter-simple sequence repeat (ISSR), expressed sequence tag-simple sequence repeat (EST-SSRs), and single nucleotide polymorphism (SNP) (You et al. 2016; Singh et al. 2020a, b). Research on sugarcane's genetic diversity, genetic mapping, paternity analysis, segregation analysis, and marker-assisted selection (MAS) has extensively used microsatellite (SSRs) markers more than the others (Ali et al. 2017; Singh et al. 2019; Malviya et al. 2020). Because SSR markers are codominant, multi-allelic, highly polymorphic, and have good genome coverage, they are regarded as the most effective markers in plant genetics and breeding programs (Aitken et al. 2005). Additionally, they may have genome-wide dispersion and codominant inheritance as hereditary traits. They are also linked to agronomic characteristics in the functional genes of the plant genome and significant alleles involved in defense, stress response, and sucrose metabolism (Cordeiro et al. 2001; Xiu-Peng Song et al. 2019; Huang et al. 2022; Joshi et al. 2023). Researchers may thus evaluate genetic diversity and population structure among *Saccharum* spp. and other grass species by employing SSR markers. The study of genetic diversity helps to understand the genetic relationships among research materials and correlations

among characteristics and features that contribute most to variety to identify genotypes and traits that are appropriate for selection and hybridization.

4.2 Microsatellites: Definition and Importance

4.2.1 What Are Microsatellites?

Microsatellites (Litt and Luty 1989), short tandem repeats (STRs; Edwards et al. 1991), simple sequence repeats (SSRs; Jacob et al. 1991), or simple sequence length polymorphisms (SSLPs; Tautz 1989) are tandem repeated motifs of 1–6 bp that are generally present in all prokaryotic and eukaryotic genomes studied to date. SSRs may be found throughout the nuclear genome in both coding and noncoding areas. These are also present in the genomes of mitochondria (Rajendrakumar et al. 2007; Guo et al. 2021, 2023) and chloroplasts (Chung et al. 2006). Based on their size, the kind of repeat unit they include, and their location throughout the genome, microsatellites have been divided into many categories. Based on the number of nucleotides per repeat unit, SSRs are classified as mono-$(A)_{10}$, di-$(CT)_5$, tri-$(CTG)_3$, tetra-$(CAGA)_4$, penta-$(AAATT)_5$, or hexa-$(CTTTAA)_6$. Microsatellites can be categorized as nuclear (nuSSR), mitochondrial (mtSSR), or chloroplast SSRs (cpSSR) according to their location in the genome. In contrast, Wang et al. (2009) classified microsatellites according to the nucleotide arrangement in the repeat motifs as perfect repeat (CTCTCTCTCTCTCTCT), compound repeat (CTCTCTCACACA), imperfect repeat (CTCTCTCTACTCTCT), and region of cryptic simplicity (GTGTACACAGT). Perfect repeats are tandem arrays of a single repeat motif, whereas imperfect repetitions include non-repeat motifs punctuated by perfect repeats at certain spots. In compound repeats, two basic patterns of repeating coexist in different configurations.

SSRs are characterized by low levels of repetition per locus (5–100), a relatively random distribution across the genome of about 104–105 SSRs per genome (Tautz 1993), and high levels of length polymorphism (Zane et al. 2002; Singh et al. 2020a, b, 2021a). The high-length polymorphism brought on by different numbers of repetitions in the microsatellite regions may be easily and consistently detected by polymerase chain reaction (PCR). SSRs are highly polymorphic because they undergo mutation at far greater rates than nonrepetitive sequences (Wu et al. 2022). Microsatellite diversity may result via gene conversion, sister chromatid exchange, uneven crossing over, and replication slippage. According to Donnelly et al. (1999), replication slippage is a key factor in producing novel alleles at microsatellite loci. High site-specific and reversible rates of increase or decrease in the number of tandem repeats of a brief DNA motif are characteristics of SSRs (Fig. 4.1).

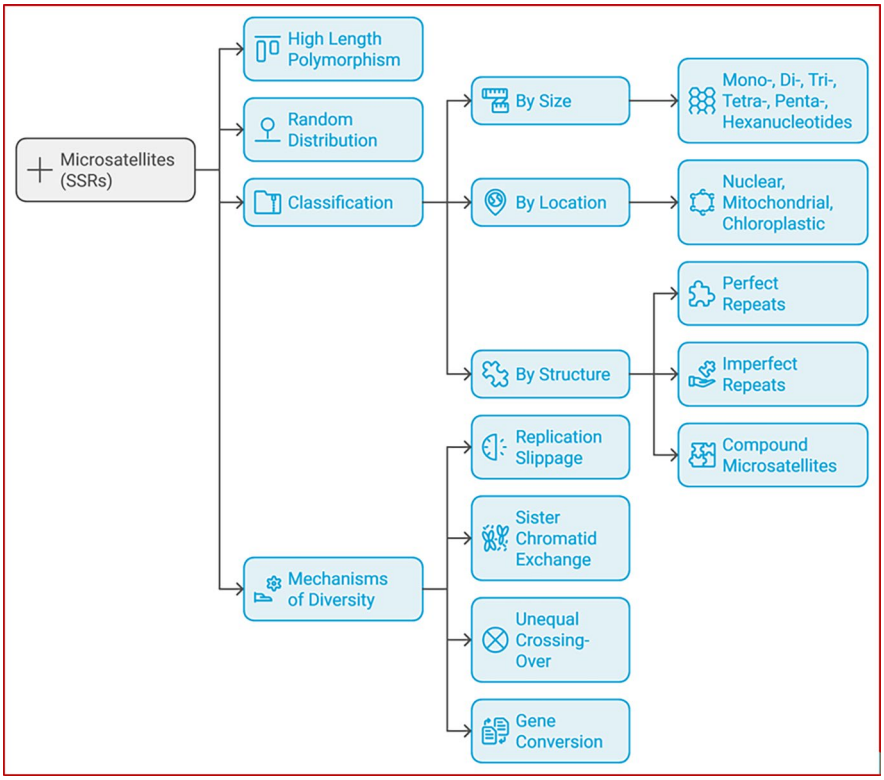

Fig. 4.1 Characterization and classification of microsatellites (SSRs). Developed using the platform at https://app.napkin.ai/

4.2.2 Importance of Microsatellites in Sugarcane Genetics

Understanding genetic diversity and the relationships within germplasm collections is essential for enhancing desired traits. In the development of sugarcane, selecting parental lines for crossing necessitates a comprehensive grasp of breeding techniques, along with careful characterization and assessment of the germplasm. In sugarcane breeding, it is common for breeders to choose parental accessions primarily based on their phenotypic characteristics, often lacking insight into their genetic relationships. Employing a strategy based on molecular markers will facilitate this process (Singh et al. 2021b, c; Joshi et al. 2022; Deng et al. 2023). SSRs are extensively preferred due to their codominance, high detection capability, abundance, and uniform distribution despite the availability of various PCR-based markers (Andru et al. 2011; Sharma et al. 2022). SSRs from closely related sugarcane species have a high degree of sequence conservation, which makes their amplification easier (Ahmad et al. 2018; Malviya et al. 2024). SSRs are a valuable tool for estimating genetic diversity in cross-species genomic studies due to their ability to

yield substantial amounts of genetic information. SSR markers are frequently utilized in sugarcane research to assess genetic variation at the molecular level.

4.3 Genetic Diversity in Sugarcane

4.3.1 Genetic Diversity in Sugarcane Varieties

The genetic diversity in modern sugarcane cultivars has been significantly influenced by hybridization origins and selective breeding techniques used throughout time. Modern sugarcane cultivars are developed through interspecific hybridization of *Saccharum officinarum* and *Saccharum spontaneum*, resulting in highly complex genomes. Consequently, ploidy levels may vary from octaploidy ($x = 8$) to dodecaploidy ($x = 12$), with hybrid progenies undergoing multiple backcrossing into *S. officinarum*. In the cultivars, *S. officinarum* comprises approximately 70–80% of the chromosomes, with *S. spontaneum* contributing 10–15% and interspecific recombination making up 5–10% (D'Hont et al. 1996). The complex polyploid characteristics of sugarcane, arising from interspecific hybridization, significantly contribute to its genetic diversity (Afghan et al. 2024; Li et al. 2024). The primary origin of sugarcane cultivars stems from the hybridization of *S. spontaneum* and *S. officinarum*, leading to a diversified genetic pool (Cursi et al. 2022) (Sforça et al. 2018). The genetic foundation of sugarcane has been enhanced through intergeneric hybridization, introducing traits such as disease resistance and increased biomass (Sundaram et al. 2010; Chen et al. 2020). A complex interaction of traits, breeding techniques, and molecular strategies characterizes the genetic diversity found in modern sugarcane cultivars (Sharma et al. 2021; Xu et al. 2022). Analysis of pedigree records and phenotypic traits has revealed genetic diversity. However, the ability of phenotypic characteristics to reveal the real extent of diversity within the sugarcane germplasm is limited by the considerable impact of environmental factors (Verma et al. 2019a, b; Oliveira et al. 2022). Modern breeding techniques utilize genomic selection (GS) to tackle this challenge, enabling breeders to utilize whole-genome data for accurate genome selection and improved traits.

4.3.2 Importance of Genetic Diversity

The genetic diversity within sugarcane plays a vital role in improving the crop's resilience, yield, and ability to adapt to environmental challenges. This enables the selection and development of exceptional cultivars capable of flourishing in diverse environments. Integrating diverse genotypes into breeding programs can improve traits such as sucrose content and disease resistance, thereby boosting yields in agriculture. The availability of a larger gene pool, facilitated by genetic diversity,

enhances the selection of desired traits for breeding programs (Malviya et al. 2021; Guo et al. 2022; Perera et al. 2023). Identifying superior genotypes exhibiting better traits suggests that genetic diversity is crucial for improving sucrose yields (Anas et al. 2021a, b; Momotaz et al. 2024). The identification of significant genetic variations among genotypes has streamlined the selection of high-performing cultivars facilitated by the application of molecular markers such as SSRs (Hasan et al. 2023). On the other hand, a decrease in genetic diversity within sugarcane can result in heightened vulnerability to diseases and diminished adaptability, highlighting the importance of continuous initiatives to preserve and improve genetic variability in breeding programs. The complex polyploid genome of sugarcane, extended breeding cycles, and the low heritability of essential traits result in gradual genetic improvements in yield and disease resistance (Song et al. 2021, 2022; Luo et al. 2023) (ElSayed and Omar 2022). The dependence on a narrow range of cultivars has resulted in diminished genetic diversity, increasing the vulnerability of crops to diseases and environmental pressures (Verma et al. 2022; Krishna et al. 2022; Chen et al. 2024). Developments in genomic selection (GS) and molecular marker-assisted selection (MAS) offer potential routes to overcome these constraints by enabling more accurate breeding techniques (Luo et al. 2023).

4.4 Microsatellite-Based Analysis of Genetic Diversity

4.4.1 Methodology for SSR Analysis

Genomic DNA can be extracted from plant tissues using a modified cetyltrimethylammonium bromide (CTAB) method, as previously described by (Ali et al. 2017). DNA quality and concentration assessment can be conducted through a ultraviolet (UV) absorbance assay. To assess DNA quality, agarose gel electrophoresis should be utilized at a concentration of 0.8–1% (Ali et al. 2017; Malviya et al. 2022). The parameters of PCR amplification, such as specificity, competence, and reliability, are significantly influenced by the components used in the DNA amplification tube and the thermal cycling conditions (Caetano-Anolles and Brant 1991; Singh et al. 2022). Therefore, effective standardization of PCR components and conditions will ultimately result in high reproducibility and strong amplification. The protocol used by Hasan et al. (2023) can be utilized for PCR amplification and gel electrophoresis. Following gel electrophoresis, one can estimate the size of each amplification product by comparing its migration pattern with that of known molecular weight markers using a DNA ladder (Fig. 4.2).

Every unique band or fragment observed on the gel can be designated an identification number according to its location and assessed visually as either present (1) or absent (0) for each individual and primer utilized. The scores for all primers were aggregated to form a data matrix. This data matrix facilitates estimating linkage distance (D) and constructing a UPGMA (unweighted pair group method of

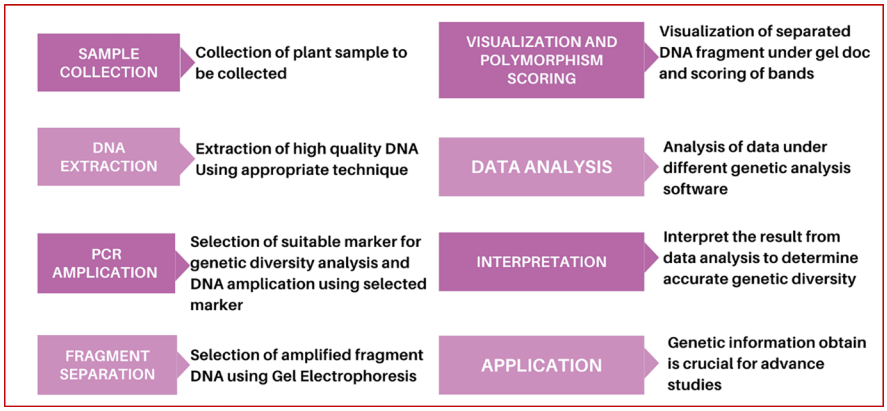

Fig. 4.2 Schematic representation of the steps involved in the analysis of genetic diversity (revised figure using the platform https://www.canva.com/design from Bidyananda et al. (2024), an open-access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https://creativecommons.org/licenses/by/4.0/))

arithmetic means) dendrogram among populations utilizing the Statistica software. Genetic similarity values, defined as the fraction of shared bands between the SSR profiles of any two individuals on the same gel, were calculated manually from the data matrix's SSR primer of the same molecular weight. Using the SSR scoring data, one can perform calculations for various genetic metrics, including the number of different alleles (Na), the number of effective alleles (Ne), Shannon's information index (I), expected heterozygosity (He), unbiased expected heterozygosity (uHe), and the polymorphism information content (PIC) value with the help of GenAlEx 6.5 software (Bayat et al. 2021). The GS matrix is utilized for cluster analysis through a straightforward agglomerative hierarchical clustering method, precisely the unweighted pair group method with arithmetic mean (UPGMA) (Singh et al. 2020a, b). Linkage distances calculated from the frequencies of polymorphic markers to assess the genetic relationship employing the unweighted pair group method of arithmetic means (UPGMA) and dendrogram can be generated utilizing the Statistica software package (Bayat et al. 2021; Bhatt et al. 2024).

4.4.2 Statistical Tools for Genetic Diversity Analysis

STRUCTURE is an openly accessible software tool designed for population analysis, created by Pritchard et al. (2000). It identifies populations from data and assigns individuals to the population that best fits the variation patterns observed. The genetic structure of all accessions can be assessed using model- and distance-based methods with the STRUCTURE analytical tool developed by Pritchard et al. (2000). Perera et al. (2023) investigated the genetic diversity and population structure of 96

Saccharum commercial hybrids utilizing the SSR marker and derived genetic similarity from the analysis. STRUCTURE utilizes a model to analyze the data from K presumed populations or genetic groups, each defined by a specific subset of allele frequencies identified within the dataset. Principal component analysis (PCA) helps visualize genetic diversity and population structure, indicating that geographical origin does not solely determine genetic relationships (Chen et al. 2023; Zheng et al. 2023). Principal component analysis (PCA) illustrated that the distribution of accessions in the scatterplot was significantly spread out, highlighting the considerable genetic diversity among accessions of various species (Li et al. 2023; Singh et al. 2023). Shahzad et al. (2022) examined 60 hybrids of *Saccharum* spp. utilizing simple sequence repeat (SSR) primers and assessing the population structure. The analysis conducted with STRUCTURE V2.3.1 software categorized the genotypes into three distinct subpopulations (Fig. 4.3).

The genetic similarity among the accessions from the data matrix was assessed using Jaccard's identity coefficient with the FreeTree software package (Hampl et al. 2001). The phylogenetic tree was constructed utilizing TreeView software based on a pairwise genetic similarity matrix (Page 1996). R Studio and various statistical packages were employed for the univariate and multivariate analyses of the data (Tolera et al. 2023). Genetic diversity was assessed, and phylogenetic trees

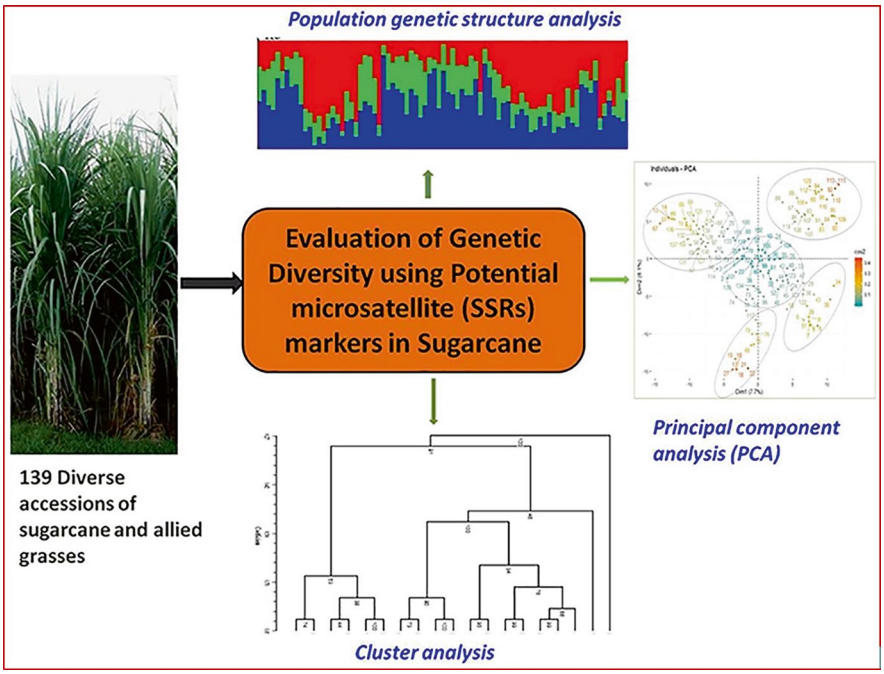

Fig. 4.3 Schematic illustration of the outcomes from various statistical tools utilized in the analysis of genetic diversity in sugarcane. (Reproduced figure from Singh et al. (2020a, b) with permission from Elsevier B.V)

were constructed utilizing MEGA 7, employing the maximum likelihood tree method as per the parameters set forth (Shi et al. 2017). To analyze the genetic relationships between accessions, a principal component analysis (PCA) can be conducted based on the variability trends of simple sequence repeats using the R program (package "ggplot2" version 3.0.0) (Singh et al. 2020a, b).

4.5 Case Studies of Microsatellite Analysis in Sugarcane

4.5.1 Global Case Studies

A study conducted by Xiong et al. (2022) evaluated the genetic diversity and population structure of 196 *Saccharum* accessions, which comprised 34 *S. officinarum*, 69 *S. spontaneum*, 17 *S. robustum*, 25 *S. barberi*, 13 *S. sinense*, 2 *S. edule*, and 36 *Saccharum* spp. hybrids. A total of 624 polymorphic SSR alleles were amplified using PCR with 22 pairs of fluorescence-labeled highly polymorphic SSR primers and identified on a capillary electrophoresis (CE) detection system, which included 109 new alleles. Three approaches were employed to analyze population structure and genetic diversity: model-based clustering, principal component analysis, and phylogenetic analysis. The findings indicated that the 196 accessions could be categorized into either 3 populations (Q) or 8 (q) subpopulations. Phylogenetic analysis revealed that the majority of accessions from each species clustered together. The species *S. barberi* and *S. sinense* constituted a single group. The species *S. robustum*, *S. barberi*, *S. spontaneum*, *S. edule*, and sugarcane hybrids were classified into the second group. The accessions of *S. officinarum* constituted the third group, positioned between the other two groups. A total of 24 species-specific or species-associated SSR alleles were identified through two-way chi-squared tests. This includes four alleles for *S. officinarum*, *S. spontaneum*, *S. barberi*, and *S. sinense*, five for *S. robustum*, and three for *Saccharum* spp. hybrids.

Singh et al. (2020a, b) conducted a study on the genetic diversity and population structure of 139 accessions of the genus *Saccharum*, employing informative microsatellite (SSR) markers. A total of 427 alleles were generated utilizing 61 polymorphic primers, with the number of alleles produced ranging from 2 to 13, averaging 7 alleles per locus. The PIC values varied between 0.35 and 0.90, yielding a mean value of 0.66 across all evaluated markers. The UPGMA analysis identified three primary clusters, which were subsequently divided into nine subclusters. The study of population structure revealed three distinct subpopulations within the utilized accession set; however, no correlation was found between these sub-groupings and their respective places of origin. Analysis of molecular variance (AMOVA) further validated that 83% and 17% of the total variations were linked to within and between populations, respectively, indicating a significant exchange of gene pool across different places of origin. Principal component analysis (PCA) revealed that the

distribution of accessions in the scatterplot was significantly dispersed, indicating a rich genetic diversity between the accessions of various species.

4.5.2 Relationship and Phylogenetic Analysis

Microsatellite loci serve as valuable tools for assessing genetic distances, constructing phylogenetic trees, and elucidating genetic relationships among various species or populations. Phylogenetic trees establish the evolutionary connections among different species or populations by assessing the genetic distances between them. Constructing these trees involves various methodologies, such as distance-based approaches, maximum parsimony, and maximum likelihood techniques. Creating phylogenetic trees using microsatellite data involves several steps. Initially, collecting microsatellite data from the species or populations of interest involves identifying and sequencing specific microsatellite loci. Subsequently, these data can be used to calculate genetic distances between individuals or populations using metrics like Cavalli-Sforza and Edwards' chord distance and Nei's standard genetic distance. From these calculated distances, a phylogenetic tree can thus be constructed utilizing methods such as maximum likelihood, unweighted pair group method with arithmetic mean (UPGMA), and neighbor joining (NJ). The phylogenetic tree undergoes a thorough analysis for accuracy and reliability, often utilizing bootstrapping methods to evaluate the strength of its topology. Finally, the tree is analyzed to understand the evolutionary connections between the species or populations, providing valuable insights into their evolutionary history and genetic variation.

4.5.3 Implications for Sugarcane Breeding

Currently, the main breeding method employed for developing sugarcane varieties is cross-hybridization. The selection of parental clones for crossing represents the most critical phase in the traditional sugarcane cross-breeding process. Only parental clones exhibiting significant complementarity and genetic diversity can generate high-quality seedling populations (Jackson 2005). Defining the genetic relationship between the parental clones utilized in the latest sugarcane cross-breeding initiatives is essential to facilitate informed crossing decisions. According to Wu et al. (2019), combining high-performance capillary electrophoresis (HPCE) with fluorescence-labeled SSR markers could be advantageous for genotyping polyploid sugarcane. By enhancing our comprehension of genetic diversity and population structure among parental lines, SSR (simple sequence repeat) analysis has significantly impacted breeding methodologies in sugarcane. SSR analysis is essential in paternity analysis, enabling breeders to reconstruct pedigrees and understand the genetic contributions of both parents. Using SSR markers, they can identify the male parent of progenies in ploycross families. SSRs are valuable for pinpointing

specific alleles that signify male parentage, proving particularly advantageous in cases where only the female parent is identified. The genetic composition and comprehension of parental lines enhance germplasm management, allowing breeders to select parents that genetically complement one another and contribute to a wider genetic foundation in the resulting seedlings. The implementation of SSR analysis in breeding strategies results in the development of enhanced sugarcane cultivars characterized by traits such as increased yield, improved disease resistance, and higher sugar content, which are crucial for satisfying the needs of the sugar and bioenergy markets. Moreover, SSRs derived from expressed sequence tags (EST-SSRs) exhibit greater interspecific transferability compared to genomic SSRs, rendering them valuable for cross-species comparisons, evolutionary research, and genetic mapping. Moreover, using SSR markers simplifies segregation analysis, which is essential for comprehending trait inheritance and selecting preferred characteristics for breeding initiatives. The application of SSR analysis is crucial for enhancing sugarcane genetic diversity and breeding efficiency, ultimately leading to the development of superior cultivars (Ahmad et al. 2018).

4.6 Challenges and Limitations of Microsatellite Analysis in Sugarcane

Developing SSR markers for polyploid crops like sugarcane presents several challenges due to the complex nature of their genomes and multiple homologous sequences, complicating the genetic analysis. The significant sequence similarity among the sub-genomes of polyploids presents difficulties in the development and analysis of molecular markers as well as in conducting sequencing studies. This complexity complicates the reliable identification of SSR loci and the differentiation of alleles. Moreover, the significant level of genetic redundancy in polyploid crops may pose challenges in developing SSR primers tailored to a specific locus. A significant number of SSR loci might be conserved among various species, potentially leading to cross-amplification and nonspecific binding in the process of PCR amplification (Zheng et al. 2023). This may add additional challenges to identifying distinct markers that can benefit breeding applications. A significant number of SSR loci in sugarcane exhibit a notable level of sequence conservation, especially EST-derived SSRs, which display lower variability than genomic SSRs. The extremely low polymorphism of EST-SSRs restricts their application in detecting minor genetic variations within populations or differentiating between closely related cultivars.

In genetic studies, the challenges of interpretability and reproducibility of SSR marker data present significant hurdles. The issues arise from several factors, such as varying methodologies, inconsistent data, and the inherent limitations of SSR markers. While SSR alleles were consistently amplified, a network experiment indicated a lack of standardization, as various laboratories may employ different

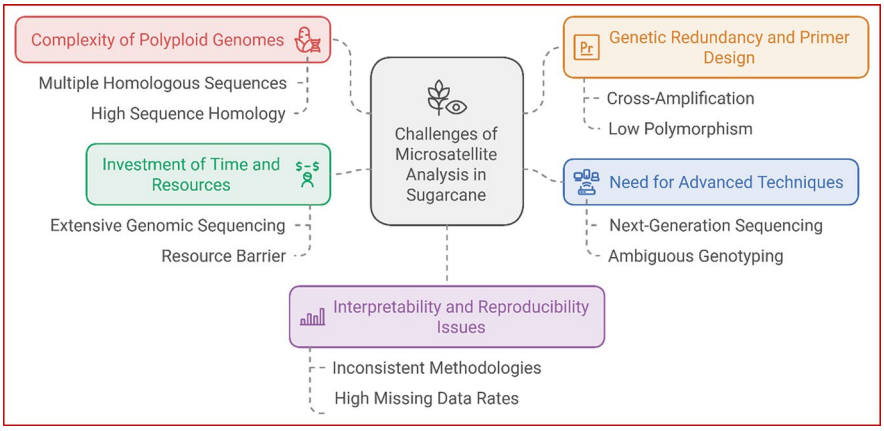

Fig. 4.4 Schematic representation illustrating the challenges associated with microsatellite analysis in sugarcane. Developed using https://app.napkin.ai/

protocols for SSR amplification, leading to minor discrepancies in sizing (Rajput et al. 2006; Jones et al. 1997). In contrast to SNP markers (2.1–3.1%), SSR markers have a more significant percentages of missing data (13.8%), which might make replication and data interpretation more difficult (Jones et al. 2007). SSRs might yield less reliable results due to their lower repeatability rate of 91.7%, compared to the 98.1% repeatability of MassARRAY SNPs (Jones et al. 2007). Due to the complexity of the models involved and the context-dependent performance of different analytical techniques, interpreting population structure and demographic history using SSR data might present challenges (Putman and Carbone 2014). Breeding advancement and genetic improvement initiatives may be slowed by this limitation, making it more challenging to identify quantitative trait loci (QTLs) or other genetic variations linked to agronomic variables (Fig. 4.4).

The challenges at hand require sophisticated techniques and technologies, including next-generation sequencing (NGS), to efficiently develop and apply SSR markers in polyploid crops. In polyploid species, individuals may possess multiple copies of a gene, resulting in a diverse array of allele combinations that can complicate the interpretation of genetic data. This may result in unclear genotyping outcomes, complicating the differentiation between genuine genetic variation and technical artifacts. Furthermore, creating SSR markers demands considerable time and resources, encompassing comprehensive genomic sequencing and detailed characterization of the species in question.

4.7 Advances in and Alternatives to Microsatellite Analysis

4.7.1 Development of EST-SSRs and Genomic SSRs

SSR markers can typically be classified into two main types: expressed sequence tag SSRs (EST-SSRs) and genomic SSRs (gSSRs). However, gSSRs originate from the broader genomic DNA. EST-SSRs specifically represent the coding regions of the genome as they arise from expressed sequences or transcripts. In contrast, SSR markers derived from EST sequences are located in the expressed regions of the genome, rendering them more functionally relevant than genomic markers (Cordeiro et al. 2001; Carson and Botha 2000). EST-SSR markers possess a notable advantage over genomic SSRs due to their swift electronic search acquisition and capacity to capture genetic variability within or adjacent to genes (Varshney et al. 2005).

The abundance of transcriptome data produced by high-throughput sequencing makes EST-SSRs in sugarcane easier to access. EST-based SSR markers play a crucial role in sugarcane studies, particularly in enhancing genetic analysis and breeding initiatives that facilitate the identification of genetic variations associated with important agronomic traits such as sugar yield and content. The genetic diversity of sugarcane cultivars can be evaluated using EST-SSR markers, which can reveal significant differences relevant to breeding programs (Xiao et al. 2020). A study demonstrated the ability of these markers to capture significant genetic diversity across various sugarcane accessions, identifying 46,043 loci of simple repeat sequences (Xiao et al. 2020). The validation of EST-SSR markers has shown their effectiveness in marker-assisted selection (MAS) for variables such as Brix value and sucrose content, which are crucial for sugar yield (Divakar et al. 2023). Although EST-SSR markers offer several benefits, there are still challenges, including the intricate nature of sugarcane's polyploidy and the requirement for thorough validation. The application of these markers in practical breeding scenarios can be complicated by these factors, highlighting the need for further investigation to enhance their effectiveness in sugarcane improvement.

The significant polymorphism, consistency, and prevalence of the plant genome enhance the reliability and attractiveness of genomic SSR markers. SSR markers derived from genomic libraries in sugarcane have demonstrated more significant polymorphism and generated extra fragments (Pinto et al. 2006; Kalwade and Devarumath 2014). The larger sugarcane genome enables the use of a higher number of genomic SSR markers, enhancing map coverage and facilitating the spanning of untranscribed regions by genomic SSRs. This feature enhances the utility of genomic SSRs in exploring genetic diversity, conducting DNA fingerprinting, and identifying true hybrids (Parthiban et al. 2018). Unlike genomic sequences that are scattered throughout the genome, EST-SSR markers are located in more conserved and expressed sequences, which could explain their relatively low level of polymorphism. Genomic SSRs are particularly helpful for classifying genotypes into discrete clusters due to their high PIC (polymorphism information content) values and low genetic similarity scores. The efficiency of genomic SSRs in differentiating

genotypes in sugarcane presents significant benefits for enhancing breeding programs and identifying diverse parental lines (Parthiban et al. 2018).

4.7.2 Comparison with Other Molecular Markers

4.7.2.1 SSRs Vs. SNPs (Single Nucleotide Polymorphisms) and Other Markers in Genetic Diversity Studies

In sugarcane genetic investigations, other markers like RAPD (random amplified polymorphic DNA) and AFLPs (amplified fragment length polymorphisms) have also been employed. AFLPs serve as valuable tools for exploring diversification in species that lack extensive genomic resources, as they exhibit high polymorphism and provide broad genome coverage without the need for prior sequence data. Nonetheless, their applicability in detailed genetic studies might be constrained due to their nature as dominant markers and lower repeatability than SSRs and SNPs. Although RAPD is simple and cost-effective, it is often limited by its lack of repeatability and sensitivity to experimental conditions, leading to inconsistent results across various laboratories and studies (Park et al. 2024). The most common markers found in genomes are SNPs, which represent single-base variations in the DNA sequence and are uniformly distributed across both coding and noncoding regions. Compared to SSRs, they provide a significantly higher density and genome-wide coverage, which is especially advantageous for large polyploid genomes like the ones seen in sugarcane. Due to the capability of automated sequencing technologies to effectively evaluate SNPs, delivering rapid and precise data for numerous samples, they are ideally suited for high-throughput genotyping. SNPs facilitate the identification of genes associated with traits such as sugar content and disease resistance, as they cover a more significant portion of the genome (Zhang et al. 2022).

Even though SNPs usually exhibit less variability per locus than SSRs, their abundance provides a more complete picture of the genome by identifying subtler genetic variations that SSRs might overlook. Over the past three decades, SSR markers have been extensively used in marker-assisted breeding, selection identification, association analysis, and plant genome mapping. During PCR amplification or due to DNA enzyme slippage and duplication, SSR markers can undergo mutations along the length of the SSR loci. This may lead to an extensive array of shutter bands when detecting polymorphism, complicating the interpretation of bands and the development of various SSR detection systems. SNPs may prove advantageous for high-throughput automatic detection because of their low mutation rate (Divakar et al. 2023) (Wu et al. 2019). The primary drawback of sugarcane SNPs is their difficulty in interpreting data because of polyploidy, given the complexity of the sugarcane genome (Park et al. 2024).

4.7.2.2 Emerging Trends: Next-Generation Sequencing (NGS)-Based Markers and Their Impacts on Genetic Diversity Analysis in Sugarcane

Next-generation sequencing (NGS) is a highly advanced sequencing technology that has transformed the field of genomic study. It has facilitated the analysis of numerous genomes to investigate diversity within or among germplasm pools. The emergence of innovative platforms utilizing long-read sequencing has significantly enhanced the efficiency of sequencing extensive and intricate genomes. Next-generation sequencing has supplanted conventional genotyping and plant breeding techniques, streamlining the processes of genetic enhancement and modification through the accessibility of a wide array of genetic variant information. The analysis of high-throughput sequencing data facilitates the investigation of evolutionary horizons and diversity, molecular breeding, soil, and agricultural metagenomics. The genomes of polyploid crops, including *Triticum aestivum*, *Gossypium hirsutum*, *Brassica oleracea*, and *Brassica napus*, were sequenced utilizing 454 and Illumina sequencing technologies (Choulet et al. 2010; Li et al. 2015).

Additionally, the sequencing of complex polyploid plant genomes, including the genomes of *Chenopodium quinoa* and *Solanum pennellii*, was conducted utilizing single molecule real-time (SMRT) sequencing and Oxford Nanopore Technologies, respectively (Jarvis et al. 2017; Schmidt et al. 2017). NGS sequencing methods are defined by their ability to have reduced cost, high speed, and better coverage than traditional sequencing methods. Genotyping by sequencing (GBS), a high-throughput sequencing-based method, has been utilized for marker identification in crop genetics (Poland et al. 2012). The progress in sequencing technologies has resulted in the creation of genome-wide high-throughput markers that are economical and adaptable. Consequently, NGS-based genotyping has emerged as a valuable tool in agricultural genomics for conducting genome selection in both model and non-model crop species, including those with large and complex genomes. Furthermore, in this scenario, genome selection can be executed without the need for a reference genome. Sequencing can identify variants like SNPs, copy number variations (CNVs), and markers linked to specific phenotypic effects. In addition, genotyping by sequencing enables the genotyping of multiple genomes in the absence of reference genomes (Marudamuthu et al. 2023).

4.8 Applications of SSR-Based Genetic Diversity Analysis in Sugarcane Breeding

Sugarcane exhibits polyploid characteristics, with genome size and structure variations observed across different genotypes. SSRs exhibit a high degree of conservation among closely related sugarcane species, facilitating their successful amplification (Aitken et al. 2005; Singh et al. 2011; Edmé et al. 2006). Evaluating

genetic diversity and establishing phylogenetic relationships in sugarcane will provide crucial information for classifying plant germplasm accessions and selecting breeding programs (You et al. 2016; Ahmad et al. 2017). EST-SSR markers are recognized as an effective breeding tool due to their ability to localize functional genes through marker association, as they can be directly linked to the genes responsible for expressing specific traits (Palhares et al. 2012).

Hasan et al. (2023) employed SSR markers to evaluate genetic diversity across eight sugarcane varieties, uncovering notable polymorphism and genetic variation. The markers successfully differentiated 56.25% of the cultivars, contributing to a deeper understanding of their genetic relationships and performance in the field. Huang et al. (2021) employed microsatellite markers to investigate genetic diversity in 23 high-sucrose sugarcane clones and 41 frequently used parents, uncovering 95.1% polymorphism and demonstrating a high genetic similarity (0.96) among populations, which is crucial for breeding initiatives. Zeni et al. (2020) employed 36 microsatellite markers to evaluate genetic diversity across 82 sugarcane accessions, uncovering a high mean similarity of 0.8536 and moderate genetic variability (Wright's F value of 0.125), which suggests effective differentiation among the accessions.

Khalid et al. (2019) conducted a study that evaluated 46 microsatellite markers across 16 sugarcane genotypes, revealing 71.34% polymorphic loci and significant genetic diversity. The analysis organized genotypes into four distinct clusters, suggesting the possibility of leveraging a variety of genotypes in sugarcane breeding initiatives. The study conducted by Xiao et al. (2020) assessed 20 EST-SSR markers to analyze genetic diversity among 48 accessions from the *Saccharum* and *Erianthus* genera. The findings indicated that Chinese sugarcane cultivars showed lower genetic divergence than cultivars from other countries, underscoring the markers' utility in diversity evaluation. Prasitsom et al. (2019) examined 14 SSR markers to assess genetic diversity in sugarcane, categorizing them into 5 distinct groups: ABA signaling pathway, protein signaling, electron transport hemoprotein, photosynthesis, and hydrogen peroxide removal. SSR markers amplify specific regions with different patterns between the groups. A dendrogram was created utilizing four markers (SSR9, SSR80, A19, and A70) associated with the drought response, the ABA signaling pathway, and the removal of hydrogen peroxide.

4.9 Future Prospects and Research Directions

Combining simple sequence repeat (SSR) analysis with high-throughput genomic tools has significantly propelled genetic studies in numerous plant species. This collaboration improves the precision of genotyping, especially in polyploid organisms, and supports thorough evaluations of genetic diversity. A novel strategy, accurate microsatellite genotyping tool based on targeted sequencing (AMGT-TS), enables precise SSR genotyping by accommodating single nucleotide variations (SNVs) in SSRs and their flanking regions, thereby enhancing alignment and genotyping

accuracy (Huo et al. 2021). The SSR-Seq method addresses allele dosage uncertainty in polyploids, yielding corrected genotypes and improving the analysis of genetic diversity (Cui et al. 2022). Advanced sequencing technologies produce vast quantities of genomic data, enabling a more efficient analysis for identifying SSR markers. Tools like MegaSSR and GMATA are specifically developed to manage extensive datasets and deliver thorough SSR analysis. EST-SSRs are regarded as having greater interspecific transferability than genomic-SSRs in sugarcane due to their origin from transcribed regions and high homologue conservation (Varshney et al. 2005). The advancement and application of innovative integrated genomic techniques and genome-wide informative simple sequence repeat (SSR) markers are crucial for the thorough analysis of quantitative trait loci (QTLs) that influence complex phenotypes as well as for enhancing genomics-assisted breeding practices (Daware et al. 2016). Combining various forms of genomic data can improve the efficiency of resource utilization. This will enable a focus on the most informative markers while minimizing redundancy. Microsatellites or SSRs continue to be selected in labs with limited resources where modern equipment are impractical, despite the rise of advanced DNA sequence-based single nucleotide polymorphism (SNP) markers.

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Chapter 5
Innovative Breeding Strategies for Enhancing Abiotic Stress Tolerance in Sugarcane

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Abstract Sugarcane (*Saccharum* spp.) is a vital crop globally, playing a significant role in sugar and bioethanol production. It significantly supports the agricultural economies of both tropical and subtropical regions. However, abiotic stresses such as drought, waterlogging (WL), heat, cold and salinity significantly hinder its productivity by disrupting physiological, biochemical and molecular processes. These stresses reduce yield and sucrose content and threaten the crop's economic viability under changing climatic conditions. Developing stress-tolerant sugarcane cultivars is crucial to ensure yield stability under changing climatic conditions. Traditional breeding approaches, though effective, are time-consuming and limited by the crop's complex polyploid genome. Progress in molecular biology and biotechnology has introduced tools such as marker-assisted selection (MAS), genome-wide association studies (GWASs) and quantitative trait loci (QTL) mapping, transgenesis and genome editing techniques like clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated protein 9 (Cas9), all of which offer promising solutions for developing stress-resilient varieties. The role of transcription factors (TFs), microRNAs (miRNAs) and antioxidant mechanisms in

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modulating stress responses is also explored. Integrating genomic, proteomic and transcriptomic data has facilitated the discovery of genes and pathways associated with stress tolerance. Incorporating wild relatives and related genera, like *Erianthus* and *Saccharum spontaneum*, further enhances the genetic diversity available for breeding. Despite the challenges posed by genome complexity, recent advancements have paved the way for developing climate-resilient sugarcane varieties. This review underscores the need for a multidisciplinary approach that combines traditional and modern tools to mitigate the impacts of abiotic stresses, ensuring sustainable sugarcane production in an era of global climate change.

Keywords Sugarcane · Adverse environmental condition · Plant growth · Breeding · Stress adaptability

5.1 Introduction

Sugarcane (*Saccharum officinarum*) is a globally significant commercial crop with extensive demand, particularly in the sugar and bioethanol industries (Vital et al. 2017; Verma et al. 2021b; Li et al. 2024). Cultivated in tropical and subtropical regions, sugarcane cultivation spans approximately 24.9 million hectares across approximately 80 countries; it contributes to 174 million tonnes of global production (OECD/FAO 2019). However, like other crops, its productivity and sustainability are increasingly threatened by the rapidly changing climate, which gives rise to abiotic stresses like drought, waterlogging (WL), extreme temperatures, and salinity that severely disrupt sugarcane growth and productivity, frequently resulting in reduced sucrose content and biomass accumulation (Song et al. 2019; Chen et al. 2020; Huang et al. 2022; Liang et al. 2024). These stresses are anticipated to worsen under present and future conditions, creating substantial ecological and economic challenges for sugarcane production worldwide (Singh et al. 2019; Verma et al. 2022a, b; Kumari et al. 2022, 2023; Jagadesh et al. 2024). With the global population predicted to reach nearly 10 billion by 2050 and rapid globalization intensifying demands, prime agricultural land is increasingly being substituted by marginal or less optimal regions (FAO 2017; Malviya et al. 2020; Krishna et al. 2022). Environmental changes exacerbate these challenges, reducing yields and overall crop production (Song et al. 2021, 2022; Wu et al. 2022; Xu et al. 2024).

Key factors such as extreme temperatures, flooding, drought and salinity are among the primary constraints to sugarcane productivity. Abiotic stresses severely affect plant growth at morphological, anatomical and physiological levels (Singh et al. 2020; Anas et al. 2021a, b; Solanki et al. 2023). These stresses disrupt vital processes such as chlorophyll synthesis, leaf development, stomatal conductance, transpiration, photosynthetic mechanisms, enzyme function and membrane stability, ultimately compromising the productivity of crops (Singh et al. 2021a, b; Guo

et al. 2021, 2022; Verma et al. 2024a, b, c). Conventional plant breeding offers a means to address abiotic stresses by developing tolerant varieties tailored to specific stress conditions. However, this approach is resource-intensive, requiring significant time, effort and cost (Chen et al. 2023; Deng et al. 2023; Verma et al. 2023a, b). Advanced breeding approaches, including trait-based selection, inheritance analysis and marker-assisted selection (MAS), modern techniques like gene editing and omics-based approaches (proteomics, metabolomics and transcriptomics) and genome-wide association studies (GWASs) provide more efficient pathways for identifying and developing stress-tolerant genotypes (Oladosu et al. 2019; Guo et al. 2023). Conventional breeding strategies have been pivotal in sugarcane improvement; however, the integration of molecular tools and advancements in biotechnology have created new opportunities to speed up the development of stress-tolerant crop varieties (Huang et al. 2024; Afghan et al. 2024). This chapter discusses about a comprehensive approach that merges cutting-edge breeding methods with genomic strategies to improve sugarcane production in unpredictable climate challenges. By synthesizing this knowledge, this chapter aims to deepen our understanding of the molecular mechanisms underlying crop modification to abiotic stress and guide future breeding and genomic interventions.

5.2 Abiotic Stresses and Their Responses in Sugarcane

5.2.1 Drought Stress

Around the world, drought is a frequent occurrence. In addition to reducing biomass production and yield, drought is known to impede cane growth and photosynthetic capacity, thereby restricting crop production and productivity (Verma et al. 2019a, b). This issue is causing cane yield losses of up to 60% several cane-growing nations. There have recently been reports of a 40% cane production loss in the Indian states of Maharashtra and Karnataka due to drought (Gujjar et al. 2021). Drought is a significant constraint in sugarcane cultivation, especially in regions with erratic rainfall or limited irrigation (Singh et al. 2021, 2022; Xu et al. 2022; Verma et al. 2024d, e). The physiology of the drought stress mechanism in sugarcane is illustrated in Fig. 5.1.

Water scarcity significantly contributes to reduced sugarcane productivity and lower sugar recovery in current context (Verma et al. 2020a, b; Joshi et al. 2022). In response to drought, sugarcane mostly rolls its leaves to decrease the surface area available for water loss and light absorption (Verma et al. 2021c, 2023c, d; Bhatt et al. 2024). Drought stress reduces stomatal conductance, disrupts photosynthesis, and diminishes sucrose accumulation (Verma et al. 2020c, d; Gujjar et al. 2021). Tolerant varieties exhibit traits like deeper rooting, osmotic adjustment through the accumulation of osmolytes (for example, proline), and enhanced antioxidant activity to combat oxidative stress (Sharma et al. 2021, 2022; Verma et al. 2024d). Root

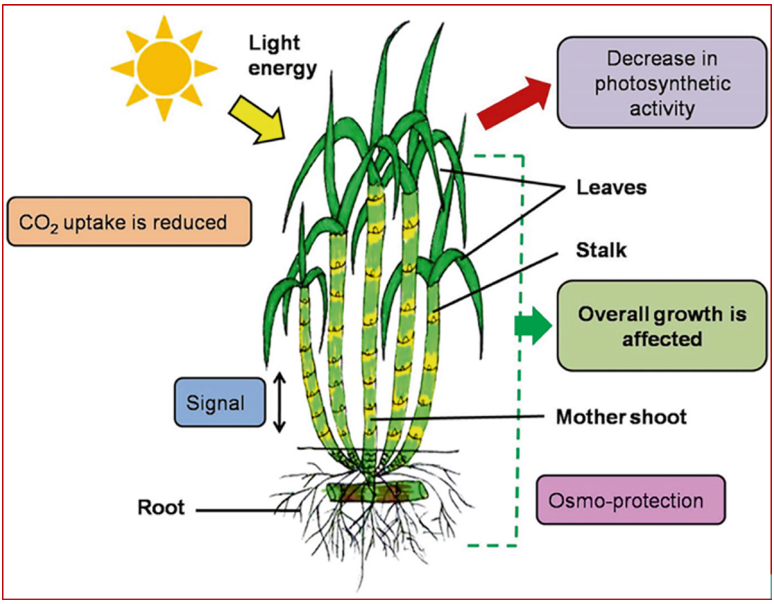

Fig. 5.1 The physiology of drought stress in sugarcane

elongation is enhanced during prolonged drought conditions, enabling plants to reach and utilize groundwater resources (Malviya et al. 2021; Verma et al. 2022c, d). Studies indicate that sugarcane is highly vulnerable to drought stress during its tillering, elongation and grand growth stages, and such stress severely affects leaf development and stem growth. Wahid (2007) reported noticeable structural damage to sugarcane leaves' chloroplast under drought conditions (Verma et al. 2020e, f). Several studies have shown that decreasing the number of green leaves on sugarcane stalks leads to reduced sucrose accumulation, as juvenile leaves play an essential role in sucrose production (Hussain et al. 2018; Hoang et al. 2019).

5.2.2 Waterlogging Stress

Between 2006 and 2016, two-thirds of the damage loss to agricultural crops worldwide was attributed to pests, with the total estimated cost reaching billions of dollars (FAO 2017). Waterlogging is common in flood-prone regions, leading to hypoxia in root systems. It disrupts nutrient uptake, root growth, and plant metabolism. A low relative growth rate, increased stalk mortality, diminished cane yield and quality deterioration are the significant impacts of waterlogging (WL) on sugarcane crops. Various factors influence sugarcane production under WL conditions, including the trial location, duration of waterlogging, water flow, the presence of aerial roots, crop growth stage, and the types of genotypes involved (Fukao et al. 2019). Waterlogging

during the cane's formative stage reduced 42.5% in biomass production, 21.63% in the tiller population, 26.52% in leaf area and 13% in plant height (Gomathi et al. 2015). Waterlogging stress lowers biomass production, dramatically decreasing leaf and stem internodal length and total dry matter (Gomathi et al. 2010). Tolerance mechanisms include aerenchyma formation and anaerobic respiration efficiency and can endure flooding conditions for an extended period (Yeung et al. 2018; You and Chan 2015).

5.2.3 Salinity Stress

Salinity affects sugarcane primarily in coastal and arid regions, resulting in osmotic stress, ion toxicity, and nutrient imbalance. Prolonged exposure to salt leads to ionic stress in plants, resulting in leaf senescence and disruptions in photosynthesis, which further adversely affect growth (Vasantha et al. 2017; Malviya et al. 2022). Extended saline stress causes roots to become heavier due to increased chloride accumulation. Reduced enzyme activity and pigment synthesis result from the photosynthetic apparatus being adversely affected by an excessive accumulation of ions, especially Na^{2+}. These circumstances slow down the rate at which carbon is assimilated. Because plants cannot use too much light, reactive oxygen species (ROS) are produced at higher levels, ultimately causing oxidative stress (Khan et al. 2019). Mechanisms like ion exclusion, vacuolar sequestration, and creation of suitable solutes (e.g. glycine betaine) contribute to tolerance (Zhang et al. 2020).

5.2.4 Heat Stress

Heat stress is becoming increasingly prevalent due to global warming. Sugarcane growth is hindered when temperatures exceed 36 °C, with the optimal temperature range between 8 °C and 34 °C (Wahid et al. 2007). In tropical regions, heat stress inhibits plant growth and development, resulting in shorter internodes, higher stem fibre content, increased node formation, and decreased sucrose concentration. Additionally, it can slow growth, cause leaf senescence, and lower rates of photosynthesis and yield (Bonnett et al. 2006). Heat stress may also hinder the germination of sugarcane fuzz (true seed) or affect sett performance, with the impact varying based on the intensity and length of the heat exposure. In sugarcane, they significantly reduce growth, yield, and the net assimilation rate, causing decreasing yield, internodal length, premature leaf senescence, and biomass accumulation (Wahid and Close 2007). Heat-tolerant genotypes exhibit protective mechanisms like heat shock proteins (HSPs) and enhanced membrane stability (Ram et al. 2007).

5.2.5 *Cold Stress*

Sugarcane is extremely sensitive to cold, and there are two primary types of cold stress: freezing stress, which occurs below 0 °C, and chilling stress, which occurs between 15 °C and 0 °C. Both types of stress present significant environmental challenges that negatively influence plant growth, development, and overall crop productivity (Xin and Browse 2000). Exposure to low and chilling temperatures limits plant growth, reduces biomass accumulation, shortens internodes, and ultimately leads to lower crop yields. However, the sucrose content in sugarcane juice is relatively unaffected by low temperatures (Clements 1980; Oliveira et al. 2022), indicating that juice quality remains stable despite adverse conditions. Freezing stress disrupts the functionality of cell membranes, denatures key regulatory proteins, and generates reactive oxygen species (Shao et al. 2007). Sugarcane grows best in an optimal temperature range of 25–35 °C, critical for achieving maximum sugar yields (Clements 1980). While frost is uncommon in subtropical regions, it can cause significant crop failures and reduce the plant's ability to accumulate sucrose in its stalks (D'Hont et al. 2008). Poor ratoon crops are frequently the result of cold damage to mature plants, which prevents younger plants from growing back. Furthermore, the plant's ability to photosynthesize is significantly hampered by exposure to cold temperatures (Inman-Bamber et al. 2010).

5.3 Biochemical Responses of Sugarcane Under Abiotic Stress

Reactive oxygen species (ROS) are produced under stress, which damages plant biomolecules and membranes and interferes with physiological functions. The antioxidant defense system is essential for scavenging ROS within plant cells to support crop development, redox signaling and plant–microbe interactions (Verma et al. 2021d, e; Li et al. 2023). Plants employ both enzymatic and non-enzymatic antioxidant mechanisms to mitigate ROS-induced damage. The enzymatic system includes ascorbate peroxidase (APX), glutathione reductase (GR), superoxide dismutase (SOD), lipid peroxidase (LPX), peroxidase (POX), glutathione peroxidase (GPX) and catalase (CAT), and non-enzymatic antioxidants consist of compounds such as ascorbate, carotenoids, tocopherols, flavonoids, glutathione, phenols and stilbenes. Among these, flavonoids have emerged as potent antioxidants in plant systems (Singh et al. 2023; Joshi et al. 2023). These antioxidant mechanisms and osmolytes neutralize ROS generated mainly during abiotic stresses, thus preventing oxidative damage to cells (Bhatt et al. 2023a, b; Zheng et al. 2023), as shown in Fig. 5.2.

The antioxidant defense system is essential to support crop growth and lessen the effects of abiotic stresses (Gupta et al. 2022). About 1–2% of plants' oxygen during aerobic metabolism is transformed into reactive oxygen species (ROS) as a by-product in organelles like chloroplasts, peroxisomes, and mitochondria. Singlet

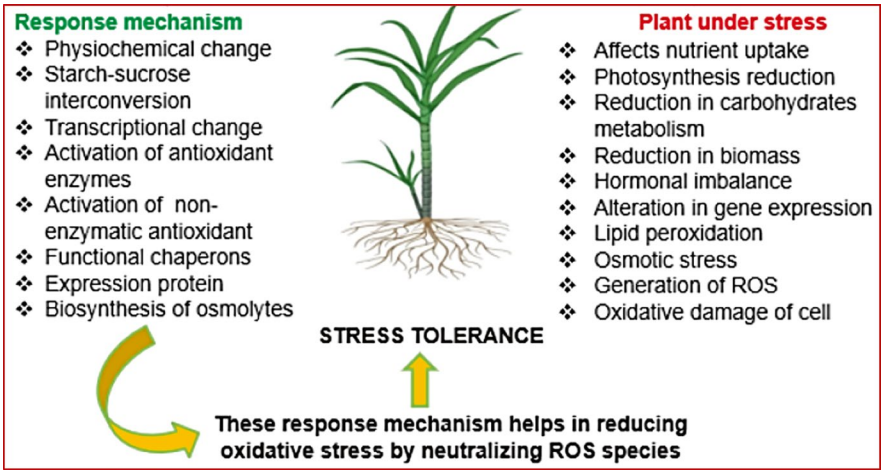

Fig. 5.2 Sugarcane's defense mechanism to withstand and fight abiotic stress-generated ROS

oxygen, hydroxyl, superoxide, hydrogen peroxide and alkoxy, and peroxy radicals are examples of ROS (Miller et al. 2010; Farnese et al. 2016). The electron transport chain and photochemical processes in plant cells are primary sources of ROS generation. ROS production increases dramatically under abiotic stress conditions (Verma et al. 2021f), particularly salinity and drought. An oxidative burst occurs when the antioxidant defense system cannot neutralize excess ROS, upset cellular homeostasis, osmotic balance and biomolecules (Segal and Wilson 2018; Tian et al. 2023; Chen et al. 2024).

Abiotic stressors affect the biosynthesis, accumulation and subsequent distribution of a wide range of phytohormones in sugarcane including abscisic acid (ABA), gibberellins (GA), cytokinins (CK), indole-3-acetic acid (IAA), salicylic acid (SA) and jasmonic acid (JA) (Eyidogan et al. 2012; Gujjar and Supaibulwatana 2019; Malviya et al. 2024). Throughout their growth cycle, sugarcane encounters various stresses and relies on their natural mechanisms, known as innate tolerance, to counteract these challenges (Verma et al. 2022e, f). Furthermore, some plants have evolved to acquire tolerance, a coping strategy for stressful situations. These processes highlight the immune-like responses of plants to stress (Gupta et al. 2022).

5.4 Conventional Breeding Strategies

Abiotic stresses like drought, waterlogging, salinity and heat are common in current climate change, which presents severe obstacles to sugarcane production and lowers yield. Despite advances in conventional breeding and genetic transformation techniques, developing stress-tolerant sugarcane varieties remains a significant obstacle. The leading causes of this are the intricate responses of plants to abiotic stress, the

complexity of the sugarcane genome and the challenge of identifying morpho-physiological traits that can be utilized in the selection process for producing commercially viable stress salt-tolerant varieties. Parental selection plays a crucial role in developing commercial cultivars, as it relies on progeny performance (Breaux 1984). Apart from cane yield and sucrose content, clones' reactions to different abiotic stressors should also be assessed as this is essential when choosing suitable parental lines in the face of changing climatic conditions. Sugarcane breeding programmes employ two primary selection strategies: family selection, which is preferred when the heritability of the family mean is higher than that of an individual plant, and individual selection, which is used when traits have high heritability (Falconer et al. 1996).

In sugarcane breeding, hybridization methods include polycrossing, which involves more than two parents, and biparental crossing, which involves two parents (Cox 2000). These techniques are primarily used to develop new recombinant clones, improved sugar content, resistance to various stresses, and high yield. Factors such as water availability, rainfall patterns and irrigation infrastructure are important considerations when selecting parents for breeding, particularly for the peninsular and North Indian plain regions. Research indicates that in sugarcane breeding, greater emphasis should be placed on selecting female rather than male lines to improve yield and sucrose content (Misra et al. 2020).

Conventional breeding, governed by Mendelian genetics, involves the transfer of traits from parent to offspring. Plant breeders cross-pollinate parental lines with the desired traits to create offspring with better qualities. This technique has been widely used to improve sugarcane varieties and increase genetic diversity (Basnayake et al. 2012). Applying stress during crucial crop stages is an efficient method for assessing abiotic stress tolerance and tracking physio-biochemical parameters at various points in time (Meena et al. 2020). Integrating the indirect selection of physiological traits into breeding programmes can aid in identifying abiotic stress-tolerant sugarcane genotypes (da Silva et al. 2012; Basnayake et al. 2015). The ground nursery stage is the first step in the multi-stage process of choosing appropriate clones. This is followed by the first clonal stage (C1), the second clonal stage (C2), and the pre-zonal varietal trial stage (PZVT). At the C2 and PZVT stages, abiotic stressors like salinity, waterlogging and drought are applied to find vigorous, high-yielding plants that thrive under specific climatic conditions (Leanasawat et al. 2021).

The low heritability of pertinent traits and the strong interactions between genotype and environment (G × E), which increase the complexity of related characteristics and genes, make it challenging to identify drought-tolerant cultivars (Cattivelli et al. 2008). While conventional plant breeding has successfully developed stress-tolerant lines in some crops, the process is often slow, labour-intensive and expensive (Ashraf 2010). Modern approaches, including crop physiology, genome-wide association studies (GWASs), marker-assisted breeding, transgenesis, gene editing, and omics technologies (proteomics, transcriptomics, metabolomics) are increasingly providing valuable insights and tools for crop improvement (Oladosu et al. 2019; Verma et al. 2022g; Budeguer et al. 2021). Although quantitative trait loci

(QTL) mapping and GWASs have proven useful in pinpointing genomic regions linked to abiotic stress-related traits, many genetic variations linked to these traits remain unidentified (Liu and Qin 2021). This highlights the pressing need to combine advanced breeding methods that use high-throughput phenotyping and multi-omics platforms to better understand how sugarcane reacts to abiotic stress and accelerate breeding progress.

5.5 Modern Breeding Strategies

5.5.1 QTL-Based Tolerance

Molecular techniques are highly effective in developing cultivars with tolerance to multiple traits simultaneously. In sugarcane, transformation mediated by *Agrobacterium* is used to introduce the *Arabidopsis* vacuolar pyrophosphatase gene (*AVP-1*), conferring tolerance to both salinity and drought stresses (Kumar et al. 2014). Recent advancements in breeding and genomic technologies have focused on enhancing genotypic performance in the presence of abiotic stress. To create sugarcane varieties that are resistant to abiotic stresses, genes from wild species and related genera must be incorporated. While QTL (quantitative trait loci) mapping has been extensively studied for traits like disease resistance, yield, and soluble solid content (Balsalobre et al. 2017), no QTL mapping related to abiotic stress tolerance in sugarcane has been reported so far. The exploration of gene and QTL sources for abiotic stress tolerance needs to accelerate, building upon previously identified resources such as the wild relative *Saccharum spontaneum* and related genera like *Narenga* spp. and *Erianthus* spp., which serve as valuable reservoirs for these traits (Meena et al. 2020). However, because sugarcane has a polyploid complex genome, it is challenging to incorporate wild and related species into breeding programmes. *Erianthus arundinaceus* and *Saccharum spontaneum* have successfully created an intergeneric hybrid (Lekshmi et al. 2017).

5.5.2 Genome Editing Approaches

Genome editing is an advanced tool that enables precise modifications in the genome by creating sequence-specific double-strand breaks (DSBs) using nucleases. Non-homologous end joining (NHEJ) is used to fix these breaks using targeted insertions, deletions or replacements in the DNA. The four main nucleases are Meganucleases (MN)-Clustered Regularly Interspaced Short Palindromic Repeat (CRISPR)-Associate Nuclease 9 (Cas9) technology, Zinc-finger nucleases (ZFNs), and Transcription Activator-Like Effector Nucleases (TALENs). An increasingly popular method for modifying the sugarcane genome is the CRISPR/Cas9 system.

It is especially beneficial due to its effectiveness and capacity to resolve transgene silencing problems in sugarcane. This technology allows for the easy manipulation of multiple functional genes associated with critical agronomic traits, even at numerous genomic sites simultaneously. Furthermore, Jung and Altpeter (2016) demonstrated the successful application of TALEN-mediated genome editing.

It has been demonstrated that TALEN-mediated mutations that target a highly conserved region of sugarcane's caffeic acid *O*-methyltransferase (COMT) greatly lower the amount of lignin in the cell wall. This reduction dramatically enhances the efficiency of converting sugarcane's lignocellulosic biomass into ethanol. However, the complex sugarcane genome, characterized by its large size, high polyploidy and aneuploidy, presents constant challenges that must be solved to fully exploit the potential of this technology. Despite these hurdles, TALEN-based genome editing could be a powerful tool, especially in regions with a strict moratorium on genetically modified (GM) crop trials and usage. This approach will likely stimulate research interest and enable the development of novel and appealing crop trait changes, thus paving the way for innovative agricultural advancements in the future.

5.5.3 Marker-Assisted Selection

Molecular markers have been extensively utilized to analyse genetic diversity among sugarcane genotypes under various abiotic stress conditions (Ming et al. 2006). Marker systems such as random amplified polymorphic DNA (RAPD), simple sequence repeats (SSRs), sequence-tagged sites (STSs), target region amplification polymorphism (TRAP), restriction fragment length polymorphism (RFLP), and inter-simple sequence repeat (ISSR) have been applied to evaluate the genetic variability of sugarcane clones in response to stresses like waterlogging, drought, cold, heat, and salinity (Fahmy-Eman et al. 2008; Sharma 2009; Hemaprabha and Simon 2012; Markad et al. 2014). Notably, drought-specific candidate genes, including indole-3-glycerol phosphate synthase and superoxide dismutase, have been identified and proposed for use in marker-assisted selection to improve drought tolerance in sugarcane (Hemaprabha and Simon 2010). In sugarcane, Kido et al. (2012) found 75,404 unigenes, 213 of which were drought-responsive and upregulated in response to drought stress. It was discovered that an SCAR (sequence-characterized amplified region) marker can successfully identify drought-tolerant sugarcane genotypes (Srivastava et al. 2012). Additionally, Khan et al. (2013) utilized sequence-specific STS primers of DREB 2 to study genetic diversity concerning drought tolerance. After two homologues of Δ'-pyroline-5-carboxylate synthase (P5CS) were cloned and characterized, SoP5CS1 was discovered as a possible marker for identifying sugarcane varieties that can withstand drought (Iskandar et al. 2014).

Transcription factors like CBF/DREB regulate gene expression by binding to drought, salt, and cold, which play a critical role in stress responses-responsive cis-acting elements. In drought-resistant sugarcane clones, 10 candidate genes,

including *GST1* (glutathione S-transferase 1), *DHAR* (dehydroascorbate reductase), *DREB* (dehydration-responsive element-binding proteins), *Prokin* (protein kinase), *CAPX* (cytosolic ascorbate peroxidase), *IGS* (indole-3-glycerol-phosphate synthase), *GSHS1* (glutathione synthase 1), *SOD* (superoxide dismutase), *LEA 3* and *PIN1* (polar auxin transport gene 1), showed upregulation, indicating their involvement in drought resistance (Hemaprabha and Simon 2012). Priji and Hemaprabha (2015) identified six putative candidate genes, *ABF2*, *CIPK14*, *LEA 3*, *MYB2*, *RD28*, *RGS1* and *SnRK2*.5, associated with the ABA-dependent pathway. These genes may play a part in transferring drought tolerance because they were present in species that could withstand drought but not in susceptible *Saccharum officinarum* clones. Additionally, a QTL mapping study with 80 genotypes tied drought-related physiological traits in sugarcane to 21 marker–trait associations using SSR markers and 11 associations using TRAP markers (Sharma 2009).

5.5.4 Role of MicroRNAs

Small non-coding RNA molecules called microRNAs (miRNAs), usually 20–24 nucleotides long, control post-transcriptional gene expression in various plant species. Numerous miRNAs implicated in stress reactions exhibit conserved functions across different plants. They mediate these responses by adjusting their levels, altering the abundance of their mRNA targets or modifying the activity and mechanisms of miRNA–protein complexes (Ferdous et al. 2015; Swapna and Kumar 2017). The way that miRNAs in plants respond to stress differs depending on the species, and, even within the same miRNA family, members can react differently to different types of stress. For example, drought conditions in bent grass upregulate and downregulate certain members of the miR319 family (Zhou and Hong 2014). Interestingly, depending on the precise real-time environmental conditions, the same miRNA in the same plant species may show distinct responses to drought.

There are not many studies examining how sugarcane's miRNA expression changes in response to stress. miRNAs linked explicitly to abiotic stress responses have only been found in a few reports (Ferreira et al. 2012; Thiebaut et al. 2012; Gentile et al. 2013). Ferreira et al. (2012) used deep sequencing of small RNAs to identify 18 miRNA families in sugarcane, seven of which showed differential expression under drought stress. Five miRNAs exhibited changes after 4 days of stress, whereas six showed differential expression after 2 days. Real-time quantitative polymerase chain reaction (RT-qPCR) further confirmed five miRNAs' expression levels.

Additionally, six precursors and their targets were identified, suggesting that sugarcane adjusts its microtranscriptome differently to deal with different stages and levels of drought stress. These reactions differ depending on one's genetic background (Gentile et al. 2013). Sugarcane's differential miRNA regulation has also been reported to be responsible for salinity stress. Research also highlights the role

of in silico methods in identifying differentially expressed miRNAs and understanding their expression patterns under abiotic stress.

5.6 Omics Technologies

Omics refers to a biotechnological approach encompassing genomics, proteomics, transcriptomics and metabolomics. Various omics technologies, particularly in relation to abiotic stress in sugarcane, are briefly explored under the following subheadings.

5.6.1 Transcriptomics

Transcriptomics focuses on analysing the complete set of mRNA produced by the genome under specific conditions, making transcriptome profiling essential for understanding responses during various stress situations. Under severe abiotic stress, plants employ various molecular strategies to overcome natural stress conditions, with transcription factors (TFs) playing a notable role. Cutting-edge transcriptomics technologies can be used to investigate these mechanisms, especially in sugarcane and other crops. Belesini et al. (2017) looked into two sugarcane varieties' transcriptomic activity, "RB855453" and "SP81–3250″, subjected to varying levels of drought stress using Illumina HiScanSQ platforms. Ascorbate peroxidase, E3 SUMO-protein ligase SIZ2, MYB, important enzymes involved in flavonoid biosynthesis like coenzyme A ligase and aquaporins were among the genes they found to be upregulated. These genes are essential for improving drought tolerance.

5.6.2 Proteomics

Proteomics is an advanced field focused on studying the proteome, providing valuable insights into the proteins present in plant cells during different growth stages under specific climatic conditions. Examining the sugarcane proteome is crucial for understanding the mechanisms underlying drought resistance. Pacheco et al. (2013) investigated the differentially expressed proteome in sugarcane roots under salinity stress across different cultivars using two-dimensional gel electrophoresis (2-DE) and liquid chromatography–mass spectrometry (LC–MS). They discovered that most proteins that accumulated in response to stress were linked to membrane stability, carbohydrate metabolism, protein protection, ROS pathways, and developmental processes. In the resistant cultivar, these proteins appeared within 2 h of exposure to salinity, while in the susceptible cultivar, their presence was observed only after 72 h of salinity stress. Salvato et al. (2019) conducted an analysis of

proteins in the nuclei of drought-stressed sugarcane stalks using filter-aided sample preparation (FASP) in combination with LC–MS/MS techniques. Their results showed that most of the 74 unique proteins identified in the control plants were associated with cell wall metabolism, indicating that this process is suppressed under drought stress.

5.6.3 Metabolomics

Metabolomics is a sophisticated approach for comprehensive profiling of every metabolite in plant cells, which has been widely used to investigate the mechanisms behind drought stress and salinity in sugarcane. Recent studies have compared sugarcane cultivars that were salt-tolerant and salt-sensitive to find secondary metabolites linked to tolerance to salinity. Chiconato et al. (2019) found that salt-tolerant cultivars retained lower Na^+ concentrations in their leaves and accumulated more proline. Vital et al. (2017) conducted metabolomics studies and discovered that in different sugarcane cultivars, salinity and drought stressors increased reducing sugars like fructose and glucose while decreasing sucrose content. Increased levels of phenolic acids, anthocyanins and flavones contribute significantly to salinity and drought resistance in sugarcane (Molinari et al. 2007; Ali et al. 2019).

5.7 Conclusion and Future Prospects

Sugarcane, a vital sugar and bioethanol production crop, faces substantial productivity challenges due to abiotic stressors, including waterlogging, heat, cold, salinity and drought. These stresses disrupt plant physiological and biochemical processes, reducing yields and quality. Conventional breeding approaches have been instrumental in addressing these challenges; however, their limitations, including time constraints and genome complexity, necessitate the adoption of advanced technologies. Some of the most noticeable effects of abiotic stress in sugarcane include a decrease in the area of leaves with a narrow width, folding, rolling, shredding and discolouration of leaves as well as a decline in the formation of tillers. Recent advancements in molecular biology, genome editing and genomics have provided strong instruments for enhancing stress tolerance in sugarcane. Techniques such as marker-assisted selection (MAS), QTL mapping, CRISPR/Cas9 and genome-wide association studies (GWASs) have shown promise in identifying and modifying the key genes associated with stress resilience. Moreover, integrating knowledge of transcription factors, microRNAs and antioxidant mechanisms has deepened our understanding of sugarcane's response to abiotic stresses. Future research should focus on expanding genetic resources, enhancing genomic tools focused on biotechnological research and adopting gene editing technologies and sustainable breeding programmes. By adopting these strategies, it will be possible to maintain sugarcane

cultivation despite climate change, ensuring its continued contribution to global food and energy security.

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Chapter 6
Sustainable Sugarcane Breeding Practices

Khushboo Jain, Ramswaroop Saini, Ayushi Malik, Avinash Marwal, and S. Alarmelu

Abstract *Saccharum* spp. is one of the most important crops in the tropics and an important plant in the agro-economy of several countries. Its production involves commercially making sugar from the juice extracted from the plant's stalks or producing biofuels from the resulting molasses. Economic sugarcane breeding is time-consuming owing to its polyploid nature, sterility of superior clones, long growth period, and complex interactions of varietal diversity with environmental factors. Improvement strategies in crops are changing to enhance sugar yield per hectare. The particular relevance of sugarcane breeding is to select genotypes that improve sucrose yield and sugar quality characteristics, enabling the plants to be more resistant to pests and diseases. Over the past decade, tropical sugarcane genotypes have been bred to improve sugarcane yield and economic viability. Generally, different parts of the world have developed sugarcane breeding programs to increase sugar productivity as well as guarantee high sugar quality. New emerging technologies play a significant role in various aspects of sugarcane breeding programs. The major ones include clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated protein 9 (Cas9), machine learning (ML), artificial intelligence (AI), metabolic engineering, RNA interference (RNAi), bioinformatics, nanotechnology, etc. Advanced molecular breeding tools and biotechnological methodologies

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are now available for targeting pest- and disease-resistant traits in sugarcane. The chief focus of this chapter is on the usefulness of a target gene/metabolite/computational approach for improving sugar yield using the abovementioned emerging technologies.

Keywords Multi-Omics · Field Phenomics · Ethical Considerations · Nanotechnology · Gene editing · Crop Improvement

6.1 Introduction

Sugarcane (*Saccharum* spp. hybrids), one of the most economically significant crops globally, has a long and rich history of cultivation, tracing back approximately 10,000 years to regions in southern Asia, including India, China, and New Guinea. Initially restricted to tropical regions, the cultivation of sugarcane has expanded into subtropical and warm-temperate areas, facilitated by the development of superior varieties such as "POJ2878," "NCo310," "Co281," "F134," and "ROC22" through targeted breeding programs (Bhatt 2020; Cursi et al. 2022). Today, sugarcane is grown in more than 100 countries and spans approximately 26 million hectares, producing nearly 1.9 billion tons of fresh cane annually. In India alone, sugarcane production is about 359 million tons, with a significant portion processed into sugar by 526 sugar mills. Sugarcane plays a dominant role in global sugar production, contributing to 75% of the world's sugar supply, while sugar beet accounts for the remaining 25%. Beyond sugar, sugarcane is a major source of bioethanol, contributing to 40% of the global bioethanol market, with a total output value estimated at $80 billion. It also serves as a source of high-value chemicals, and its processing generates several by-products that have applications in ethanol production, animal feed, and bioenergy, further enhancing its economic importance (Singh et al. 2019; Que et al. 2024; Lu et al. 2024).

Most of the modern sugarcane varieties are the result of interspecific hybridization between *Saccharum officinarum* and *Saccharum spontaneum*, which has resulted in a complex genome characterized by polyploidy and aneuploidy, with chromosome counts ranging from 80 to 120. The breeding cycle for sugarcane typically extends 10–15 years, making the process of genetic improvement labor-intensive and time-consuming. As a tall perennial crop, sugarcane poses significant challenges in phenotyping, which is further complicated by its long growth cycle of 10–14 months. Additionally, the genetic pool of wild germplasms remains underutilized, and breeding technologies have not advanced at the same pace as those for other crops like rice, maize, and soybean.

The slow pace of sugarcane breeding, coupled with the challenges posed by its complex genome, underscores the need for more advanced breeding technologies (Singh et al. 2019). Crop breeding has always been central to agriculture, aiming to

improve yield, quality, disease resistance, and adaptability. Traditional breeding practices date back to the origins of agriculture, with early farmers selecting and saving seeds from the best-performing plants. Over time, with the increasing demand for higher crop yields and quality, breeding methods have evolved significantly. These advancements are driven by the need for higher production and the economic pressures of feeding a growing global population. However, the intricate nature of sugarcane's genome and its associated traits presents unique challenges. Traditional breeding methods struggle to keep pace with the growing global demand for sugar and bioethanol, particularly as the complexity of sugarcane's genome and its polyploidy complicate genetic analysis.

Sugarcane breeding has progressed through four major phases: initial domestication (Breeding 1.0), conventional breeding (Breeding 2.0), molecular-based breeding (Breeding 3.0), and smart breeding (Breeding 4.0) (Fig. 6.1). In current breeding programs, selection is carried out through clonal selection and manual screening for key traits such as cane stalk weight and sugar content, with disease resistance being addressed in the later stages. Despite advancements, the rate of genetic improvement in sugarcane has remained comparatively slow, particularly when compared to other major crops, and, in some cases, breeding progress appears to have plateaued. Contributing factors include the lengthy breeding cycle, the complexity of phenotyping in a crop of such large biomass, and the low heritability of economically significant traits. The polyploid genome's high

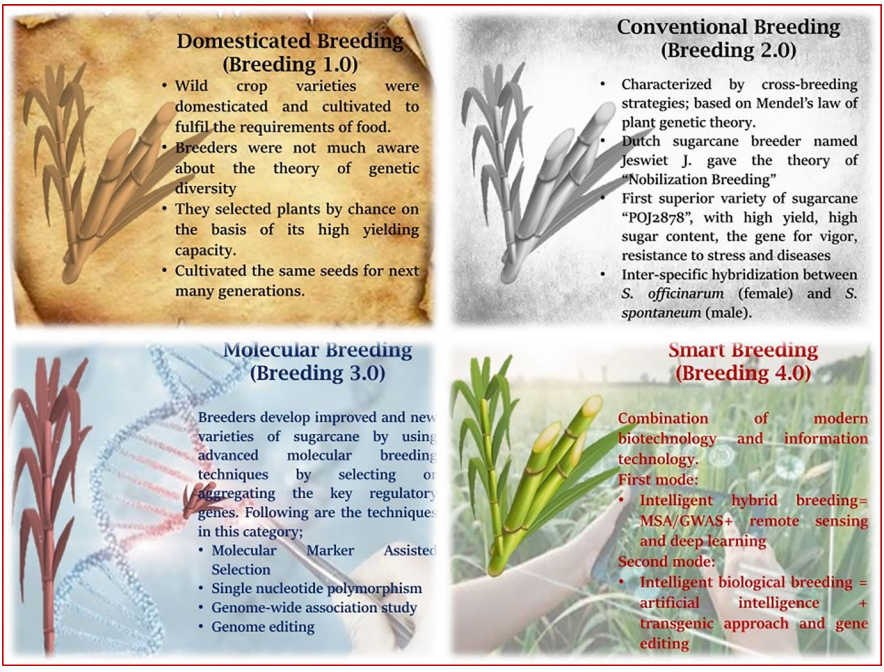

Fig. 6.1 Different phases of sugarcane breeding

heterozygosity and genotype–environment interactions further complicate the process. As sugarcane plants grow tall for extended periods, accurately assessing traits like yield potential in early-stage breeding trials is particularly challenging due to the limited space in small trial plots. These issues highlight the need for more efficient and cost-effective high-throughput phenotyping techniques, enabling more precise estimates of yield potential and facilitating faster, more accurate genetic improvement. Such advancements in breeding technology are critical for meeting the growing demand for sugarcane-derived products in a rapidly changing global agricultural landscape (Kuriakose et al. 2020; Zhang et al. 2023; Xu et al. 2022; Chandra et al. 2024).

Historically, sugarcane breeding has primarily relied on conventional methods, such as visual clonal selection and manual screening for key traits like cane stalk weight and sugar content, with breeding cycles spanning over a decade. Over time, these traditional approaches have evolved, incorporating molecular-based breeding techniques such as marker-assisted selection (MAS) and genomic tools to accelerate the development of improved varieties. The field has recently embraced "smart breeding," which integrates advanced technologies like genomic selection, high-throughput phenotyping, and computational models. The latest innovations, particularly the application of machine learning (ML) and artificial intelligence (AI), are now playing a crucial role in enhancing breeding efficiency. AI and ML algorithms can process vast amounts of genomic, phenotypic, and environmental data to predict the performance of sugarcane clones with unprecedented accuracy, enabling breeders to make data-driven decisions that accelerate genetic improvements.

This book chapter explores emerging technologies in sugarcane breeding, focusing on cutting-edge advancements such as clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated protein 9 (Cas9) gene editing, genomic selection, machine learning, and AI-driven breeding platforms. It will explore how these technologies transform breeding by enabling faster development of high-yielding, disease-resistant, and climate-resilient sugarcane varieties. Furthermore, this chapter will highlight the role of digital phenotyping and computational tools in overcoming traditional limitations, ultimately providing a roadmap for the future of sugarcane breeding in the context of global challenges such as food security and climate change.

6.2 Emerging Dry-Lab Technologies in Sugarcane Breeding

The advancement of plant breeding has significantly contributed to the improvement of agricultural productivity. Conventional breeding methods, which primarily rely on phenotypic selection, have proven to be limited in their effectiveness due to the substantial influence of environmental factors on phenotypic traits. This variability complicates the identification and selection of breeding materials for crop enhancement. With the advent of high-throughput sequencing technologies, a more detailed understanding of the genomic architecture of various crops, including

sugarcane, has been achieved. This has enabled the shift from phenotype-based to genotype-based breeding approaches, ushering in a new era of genomics-driven crop improvement (Krishna et al. 2023). The integration of dry-lab technologies such as bioinformatics, phenomics, and machine learning (ML) has further accelerated the breeding pace by optimizing the selection process and improving the precision of breeding programs.

6.2.1 Bioinformatics and Data Analysis

Bioinformatics has become a cornerstone of modern plant breeding, enabling the analysis of large-scale genomic data to identify key genetic markers and support genomic selection (Prajapat et al. 2011. The advances in sequencing technologies, coupled with powerful computational tools, have revolutionized crop improvement by facilitating the identification of genes linked to critical agronomic traits such as yield, stress tolerance, and disease resistance, especially for sugarcane (Marwal and Gaur 2020a; Nehra et al. 2019). However, the integration of bioinformatics into plant breeding presents challenges, including the management of vast datasets, the integration of multi-omics data, and the interpretation of complex biological information. Personalized breeding strategies and precision agriculture are promising outcomes of these efforts, offering the potential to tailor crop varieties to specific environmental conditions and farming practices by integrating genomic and phenotypic data (Thriveni et al. 2024).

Genomic prediction models, such as genomic best linear unbiased prediction (G-BLUP) and ridge regression best linear unbiased prediction (RR-BLUP), are extensively utilized in crop genome selection. RR-BLUP is particularly effective when many loci with small effects control traits, as it assumes equal variance across all genetic markers. G-BLUP, on the other hand, leverages genome-wide marker data to predict the genetic and phenotypic values of breeding candidates, making it a powerful tool for accelerating genetic gains in sugarcane breeding (Wang et al. 2015; Xu et al. 2022). Transcriptomic and proteomic analyses have provided insights into the molecular mechanisms underlying stress tolerance and adaptability (Maheshwari et al. 2023; Mishra et al. 2024).

Transcriptomic studies are particularly valuable in identifying genes involved in stress responses, such as drought or disease resistance (Wang et al. 2020). Notable genomic resources, including the draft genome of "SP80–3280" and the allele-defined genome of *S. spontaneum*, have been developed, though the complete genome sequence for sugarcane remains incomplete. Consequently, continued transcriptome sequencing is essential to complement existing genomic data and enhance breeding efforts for stress resilience in sugarcane (Zhang et al. 2018; Garsmeur et al. 2018). Bioinformatics tools used in transcriptome data analysis, such as Fast QC, HTQC, and PRINSEQ, are critical for ensuring high-quality raw sequencing data. These tools assess read quality, eliminate contaminants, and ensure that only

reliable data are used for downstream analysis, thereby ensuring the integrity of genomic studies in sugarcane (Schmieder and Edwards 2011; Yang et al. 2013).

6.2.2 *Machine Learning and Artificial Intelligence in Sugarcane Breeding*

The integration of high-throughput genomic platforms (HTGPs) and phenotypic data collection technologies (high-throughput phenotyping platforms; HTPPs) has expanded the capacity to dissect the biological complexity of crops like sugarcane. Noninvasive imaging techniques, such as thermal, digital, and spectroscopic imaging, as well as chlorophyll fluorescence, are invaluable for capturing phenotypic data that inform plant growth dynamics and responses to stress. With advancements in both plant phenotyping and genotyping, artificial intelligence (AI) and machine learning (ML) applications have become increasingly important in modern breeding programs, driving precision agriculture strategies that improve efficiency and productivity. AI refers to the simulation of human-like intelligence in machines, enabling them to perform tasks typically requiring human cognition, such as decision-making and pattern recognition. Machine learning, a subset of AI, involves algorithms that allow machines to learn and improve from data without explicit programming. These technologies have proven to be highly effective in analyzing large datasets and generating predictive models for crop improvement. In sugarcane breeding, AI and ML are applied to manage complex phenotypic and genomic data, develop models that predict genotype–phenotype–environment interactions, and optimize breeding decisions (Pauli 2015; Frey 2018).

ML algorithms, including artificial neural networks (ANNs) and support vector machines (SVMs), are increasingly used to forecast crop yields, improve breeding selection, and detect disease. For instance, Murali et al. (2020) developed a hybrid ML model combining statistical and neural network approaches to improve sugarcane yield forecasting. Similarly, ANN models have been used to predict sugarcane yield in India, and SVM algorithms have been employed for similar purposes (Kumar et al. 2015; Kudagi et al. 2020). In sugarcane breeding, ANNs have also been successfully applied to enhance selection within the best-performing families, demonstrating their potential to assist breeders in making more informed and accurate selections. In disease detection, deep learning approaches have shown great promise in accurately identifying diseased sugarcane plants. For example, Srivastava et al. (2020) utilized a deep learning model to classify sugarcane crops as diseased or healthy with high accuracy. Other studies have employed AI techniques for detecting plant diseases using discrete wavelet transforms (Reddy et al. 2017), while deep learning-based algorithms have been used for tasks like stem node recognition, significantly improving harvesting efficiency (Chen et al. 2021).

6.2.3 Field Phenomics and Remote Sensing

The advent of field phenomics, supported by noninvasive imaging techniques, has revolutionized the way sugarcane phenotypes are studied. Tools such as unmanned aerial vehicles (UAVs) equipped with advanced cameras are a cost-effective means to rapidly assess complex traits, including shoot/root growth, plant architecture, and greenness (Arun Kumar et al. 2020; Golzarian et al. 2011). In combination with infrared thermal imaging, UAVs have been particularly useful for identifying drought-tolerant genotypes and assessing plant health under abiotic stresses (Moller et al. 2006; Talamond et al. 2015).

Using UAVs in sugarcane breeding has facilitated canopy temperature measurement and is strongly correlated with yield potential under water-limited conditions. Studies have demonstrated that canopy temperature depression (CTD) in sugarcane correlates positively with cane yield (Basnayake et al. 2016). UAVs equipped with near-infrared or multispectral cameras can also assess traits such as leaf area index, water content, and nutrient status, providing breeders valuable insights into plant health and performance under various environmental conditions (Zhang et al. 2021). Integrating UAV-based high-throughput phenotyping has proven effective in selecting high-performing clones early in the breeding cycle. Natarajan et al. (2019) demonstrated using UAVs to monitor sugarcane growth and identify early-stage yield-related traits with a high correlation to later yield potential, thereby improving clonal selection efficiency. Furthermore, innovative models, such as the "wonder cane model" developed by Tanut et al. (2021), have enabled accurate preharvest yield forecasting using UAV imagery, offering significant benefits for farmers and the sugarcane industry.

6.2.4 Big Data Management in Plant Phenomics

The high-throughput phenotyping process generates large datasets that require sophisticated data management systems. Managing big data efficiently is crucial for the success of phenomics in crop breeding, especially under changing climate conditions. Reliable, automated data processing platforms have been developed to handle these vast amounts of information, enabling better decision-making and accelerating the breeding process. As sugarcane breeding programs continue to generate large volumes of phenotypic and genotypic data, multidisciplinary teams will be essential for effectively managing these datasets to maximize productivity and resilience in future crops.

6.3 Emerging Wet-Lab Technologies in Sugarcane Breeding

6.3.1 CRISPR/Cas9 Technology in Sugarcane Improvement

CRISPR/Cas9 has emerged as a transformative tool in plant biotechnology, including sugarcane breeding. This genome editing technology offers several advantages, such as simplicity, high target specificity, cost-effectiveness, and ease of use, making it a powerful alternative to other genetic modification techniques. CRISPR/Cas9 consists of two primary components: the Cas9 protein, which acts as a molecular "scissors," and a single guide RNA (sgRNA), which directs Cas9 to the specific location in the genome to create a double-strand break (DSB) (Fig. 6.2). The precision of this system is determined by the recognition of a 3' NGG sequence, known as the protospacer adjacent motif (PAM), which ensures that Cas9 only cuts the intended DNA sequence. Once the DSB is made, the plant cell attempts to repair the break via one of two mechanisms: nonhomologous end joining (NHEJ), which may introduce insertions or deletions leading to gene knockout, or homology-directed repair (HDR), which can facilitate precise genetic modifications, such as gene editing or the insertion of new sequences. CRISPR/Cas9 has been employed to address various challenges in sugarcane breeding, such as improving disease resistance, increasing yield and sugar content, enhancing biofuel production, and boosting stress tolerance (Singh et al. 2019).

For instance, Laksana et al. (2024) applied CRISPR/Cas9 to reduce lignin content in *Saccharum officinarum*, a sugarcane species. Lignin, an important structural component in the plant's biomass, poses challenges in bioethanol production by making the biomass harder to break down. By targeting the "*SoLIM*" gene, which regulates lignin biosynthesis, the researchers significantly reduced lignin levels, improving sugar extraction efficiency. In their study, sugarcane plants with edited "*SoLIM*" genes showed a decrease in lignin content by up to 51.46% compared to wild-type plants.

Similarly, other studies have targeted genes like acetolactate synthase (*ALS*) to induce herbicide resistance (Oz et al. 2021) and the magnesium chelatase subunit 1 (*MgCH*) gene to alter photosynthesis and enhance plant stress tolerance (Eid et al. 2021). These advancements highlight the potential of CRISPR/Cas9 as a precise and efficient tool for improving sugarcane traits, paving the way for more sustainable and resilient cultivation.

6.3.2 Nanotechnology Applications in Sugarcane Breeding

The rapid pace of global population growth and climate change challenges the traditional agricultural models established during the Green Revolution. Nanotechnology offers an innovative solution to many of these challenges, particularly in sugarcane breeding, by enabling precise pest management, nutrient delivery,

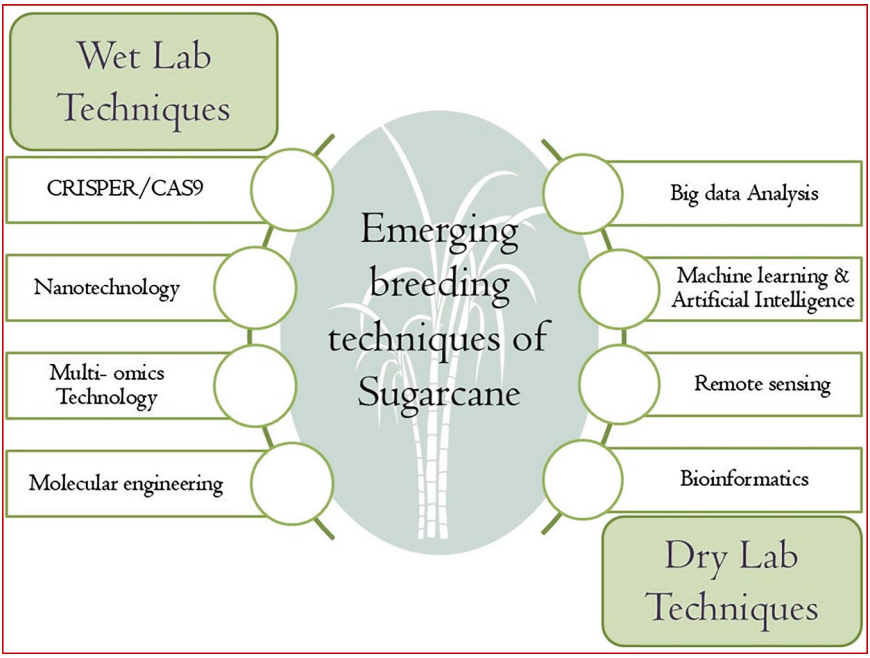

Fig. 6.2 Smart breeding techniques for sugarcane cultivation

and stress alleviation (Lowry et al. 2019; Hofmann et al. 2020). Nanomaterials, such as nano-fertilizers, nano-pesticides, and nano-sensors, are designed to optimize plant growth, improve nutrient use efficiency, enhance stress resilience, and enable early disease detection (Giraldo et al. 2019; Marwal and Gaur 2019; Kamle et al. 2020). Nano-fertilizers provide controlled nutrient release throughout the sugarcane plant's growth stages, ensuring that nutrients are delivered when needed most. These technologies help reduce nutrient loss, improve soil health, and minimize environmental impact (Raliya et al. 2017). Nano-pesticides, which encapsulate active pesticide ingredients in nanocarriers, offer more efficient pest control, as they enable targeted delivery to the pests, reduce pesticide waste, and minimize harmful residues in the environment (Usman et al. 2020; Liu et al. 2021).

Additionally, nano-biosensors, combining nanotechnology with biosensor technologies, are being used to monitor plant health, detect pathogens, and gather environmental data (Singh et al. 2021). These devices can provide real-time insights into soil conditions, pest infestations, and stress responses, which can guide future breeding strategies (Usman et al. 2020). Research has shown promising results in the application of nanotechnology in sugarcane cultivation. For instance, Elsheery et al. (2020) demonstrated that nanoparticles, such as silicon dioxide (SiO_2), zinc oxide (ZnO), and selenium (Se), could mitigate chilling stress in sugarcane plants by improving photosynthetic efficiency and protecting against photodamage. Additionally, Shi et al. (2023) showed that selenium nanoparticles (nSe) reduced the

incidence of leaf scald disease caused by *Xanthomonas albilineans*, enhanced antioxidant activity, and improved sugarcane resistance to environmental stresses. These findings underscore the potential of nanotechnology to enhance the resilience and productivity of sugarcane in an increasingly unpredictable climate.

6.3.3 Multi-omics Technologies in Sugarcane Research

Sugarcane's complex polyploid genome and large size have historically challenged its genetic study. However, integrating multi-omics approaches has opened new avenues for understanding sugarcane genetics and improving its breeding. Omics technologies—including genomics, transcriptomics, proteomics, and metabolomics—provide a holistic view of the plant's molecular processes and environmental interactions, facilitating more precise and targeted breeding strategies. Genomic studies, including genome-wide association studies (GWASs), have been used to identify genes associated with desirable traits such as high sugar content, disease resistance, and drought tolerance (Khueychai et al. 2015). Through RNA sequencing, transcriptomics allows for the comprehensive analysis of gene expression patterns, offering insights into the genes involved in various biological processes (Augustine et al. 2015). These gene expression profiles can serve as markers for developing transgenic sugarcane varieties with improved traits. Proteomics and metabolomics also play critical roles in sugarcane breeding. Proteomic techniques, such as two-dimensional gel electrophoresis (2D-DIGE), identify and quantify proteins involved in stress responses, growth, and development (Barnabas et al. 2015). Similarly, metabolomic profiling can identify the key metabolites influencing sugarcane's response to environmental stressors such as drought or nutrient deficiencies. Integrating these multi-omics data helps researchers develop new varieties that are more resilient to biotic and abiotic stresses and have higher yields and improved sustainability for biofuel production (Alwala et al. 2008; Marwal et al. 2014, 2020).

6.4 Biotechnological and Metabolic Engineering in Sugarcane Breeding

Sugarcane, an economically vital crop, is increasingly being improved through biotechnological and metabolic engineering techniques. Due to its complex genome and the challenges posed by changing climatic conditions, conventional breeding methods are often insufficient to develop new, high-performing, and resilient sugarcane varieties. Biotechnology offers precise and practical tools to introduce new traits, such as disease resistance, drought tolerance, and herbicide resistance, by directly modifying the sugarcane genome. Genetic engineering approaches have enhanced plant resistance to pests and diseases (Mahmood et al. 2017; Lakra et al.

2019). For example, introducing *Bacillus thuringiensis* (Bt) Cry genes, such as *Cry1A* and *Cry1Ab*, has conferred resistance to insect pests, improving yield stability (Gao et al. 2016). Similarly, the transformation of sugarcane with herbicide-tolerant genes, such as the glyphosate-resistant "*EPSPS*" gene, has allowed for more efficient weed management (Wang et al. 2017). In addition to genetic engineering, metabolic engineering is employed to optimize specific metabolic pathways in sugarcane. For example, metabolic engineering has been used to enhance sucrose accumulation, improve biofuel production, and increase the yield of other valuable biochemical products. Luo and colleagues (2022) applied metabolic engineering in "energy cane," a bioenergy crop, by manipulating multiple genes to promote lipid accumulation, resulting in higher ethanol production from biomass.

Molecular marker-assisted breeding also plays a critical role in accelerating sugarcane breeding programs by enabling the early selection of plants with desirable traits such as high sucrose content and disease resistance (Marwal and Gaur 2020b). Researchers use a variety of molecular markers, including simple sequence repeats (SSRs), single nucleotide polymorphisms (SNPs), and amplified fragment length polymorphisms (AFLPs), to identify these traits in sugarcane germplasm (Alwala et al. 2008; Manimekalai et al. 2020). Biotechnology and metabolic engineering offer powerful tools for enhancing sugarcane productivity, improving disease and pest resistance, and supporting sustainable agricultural practices in the face of global environmental challenges.

6.5 Ethical Considerations in Using Emerging Technologies for Sugarcane Breeding

The rapid evolution of emerging sugarcane breeding technologies, encompassing wet-lab tools like CRISPR/Cas9 and nanotechnology and dry-lab techniques such as multi-omics approaches, presents transformative opportunities to enhance crop yields, resilience, and sustainability. However, these innovations also raise critical ethical concerns that must be carefully navigated to ensure equitable access, transparency, and accountability across the agricultural value chain. These ethical issues are particularly important when considering the implications for smallholder farmers, who often remain marginalized in the evolving landscape of digital agriculture and biotechnological advancements.

6.5.1 Data Ownership, Privacy, and Transparency

One of the most pressing ethical concerns in the use of emerging technologies in sugarcane breeding is the governance of agricultural data. The collection, processing, and sharing of vast amounts of genetic, environmental, and agronomic data

generate new challenges around data ownership and privacy (Devare et al. 2021). Smallholder farmers, particularly in developing regions, often face difficulties in realizing the full benefits of technological advancements due to imbalances in data sharing. Large corporations or tech providers frequently control access to data. At the same time, farmers may be excluded from decisions about how their data are used, leading to concerns about exploitation and data misuse. The use of CRISPR/Cas9, for example, allows for precise genetic modification and generates valuable genomic data that can be leveraged to improve sugarcane traits such as disease resistance or sugar content. However, without proper regulation, there is the risk that these data could be commodified, with large corporations profiting from genetic modifications without fair compensation for farmers who provide the genetic resources. Ensuring transparency in data collection processes and clear, enforceable data ownership rights is essential to mitigate these risks. Moreover, farmers may be hesitant to share data due to concerns about data privacy, particularly in light of rising fears over the misuse of genetic information and proprietary breeding data. Establishing robust legal frameworks for data ownership and privacy is essential to protect the rights of smallholder farmers, ensuring that their contributions to breeding programs are acknowledged and that they are not exposed to unnecessary risks (Singh et al. 2021).

6.5.2 Equitable Access to Technology

Another ethical challenge involves equitable access to the benefits of emerging breeding technologies. While large-scale commercial farms have the resources to integrate technologies such as CRISPR gene editing and nanotechnology, smallholder farmers may face significant barriers, such as high costs, lack of technical expertise, and limited access to advanced tools. Adopting wet-lab technologies, such as CRISPR/Cas9 and nanotechnology-based fertilizers or pesticides, can significantly increase productivity and resilience to pests, diseases, and climate stress (Marwal et al. 2022; Verma et al. 2024). However, these technologies could exacerbate inequalities within the agricultural sector without equitable access, leaving smallholders further behind (Lowry et al. 2019). Moreover, excluding farmers from the decision-making processes related to the design, governance, and implementation of these technologies can perpetuate power imbalances. Effective technology transfer mechanisms and inclusive governance models are crucial to ensure that smallholder farmers benefit from these advances. This includes involving farmers in discussions about implementing breeding technologies and addressing their concerns about the potential risks, costs, and benefits (Hofmann et al. 2020).

6.5.3 Impact on Traditional Agricultural Practices

Introducing genetically modified sugarcane varieties and new agro-technological practices may have unintended consequences for traditional agricultural systems. While innovations like CRISPR/Cas9 offer precise gene edits to improve sugarcane traits, the rapid adoption of these technologies may threaten the knowledge and practices of indigenous and local farming communities. These communities often rely on centuries-old practices adapted to their specific environmental conditions and cultural contexts. The imposition of high-tech solutions without respect for these practices could lead to biodiversity loss, erosion of traditional knowledge, and disruption of local agricultural ecosystems. As such, ethical considerations must extend beyond the direct impact on crop yields and productivity to include the broader social, cultural, and ecological effects. Incorporating participatory breeding programs that respect traditional agricultural knowledge and local adaptation strategies can help ensure that modern biotechnological innovations complement, rather than replace, conventional farming systems (Gianotto et al. 2019).

6.5.4 Long-Term Environmental and Health Implications

Emerging technologies, such as nanotechnology, have the potential to revolutionize crop management by offering targeted delivery of nutrients and pesticides. However, nanomaterials' long-term environmental and health implications—such as nanoparticle-based fertilizers and pesticides—remain largely unknown (Tailor et al. 2022). The potential for nanomaterials to accumulate in the soil, water, and food chain raises concerns about their sustainability and impact on ecosystem health (Raliya et al. 2017). The ethical responsibility of researchers and companies involved in developing these technologies is to ensure rigorous testing and regulation to assess the safety of such materials before their widespread adoption. Furthermore, gene editing technologies like CRISPR could lead to unintended ecological consequences if modified sugarcane varieties cross-pollinate with wild relatives or nonmodified crops. These environmental risks underscore the importance of applying the precautionary principle in deploying genetically modified crops. Striking a balance between the benefits of increased productivity and the potential risks to biodiversity and ecosystem health is critical in the ethical use of such technologies (Waterworth et al. 2011).

6.5.5 Inclusive Governance and Stakeholder Engagement

The successful and ethical application of emerging technologies in sugarcane breeding relies heavily on inclusive governance models prioritizing stakeholder engagement. Smallholder farmers must be involved in designing and governance breeding programs, ensuring their concerns are addressed and their rights are respected. Effective engagement can help build trust between farmers and technology providers, encouraging collaboration and fostering an environment where all stakeholders have a say in the direction of agricultural innovations (Devare et al. 2021). At the same time, not legally binding, agricultural codes of conduct can play an important role in guiding the ethical application of emerging technologies. These codes can provide a framework for best practices in data management, stakeholder engagement, and the responsible use of genetic resources. By fostering a culture of transparency and accountability, such frameworks can promote fairness and equity in the agricultural technology sector (Singh et al. 2021).

6.6 Future Scope

Looking ahead, the future of sugarcane breeding lies in the continued integration and refinement of emerging technologies across both wet-lab and dry-lab domains. The ongoing advancements in gene editing technologies such as CRISPR/Cas9 will likely open new frontiers for developing sugarcane varieties with enhanced traits, such as improved drought tolerance, higher biofuel production, and better resistance to emerging pests and diseases. These genetic improvements will be critical in addressing the challenges posed by climate change and the increasing global demand for renewable energy sources. Furthermore, the application of nanotechnology in sugarcane breeding holds tremendous promise for optimizing crop management practices (Tailor et al. 2021, 2024). Nano-fertilizers and nano-pesticides, with their controlled and targeted delivery, could significantly improve nutrient use efficiency and pest control while minimizing environmental impact.

In the coming years, we may witness the development of more eco-friendly and sustainable nanomaterials and their integration into precision agriculture systems to monitor and manage crop health in real time. The future scope of dry-lab technologies, particularly the multi-omics approaches, will continue to play a pivotal role in deepening our understanding of sugarcane's genetic architecture and metabolic processes. The combination of genomics, transcriptomics, proteomics, and metabolomics will offer unprecedented insights into the molecular basis of the key traits, enabling the development of more efficient breeding strategies. Additionally, advancements in bioinformatics and computational biology will accelerate the discovery of novel genetic markers for disease resistance, yield improvement, and climate resilience. As the field of sugarcane breeding progresses, it is essential to maintain a strong focus on the ethical considerations discussed earlier. The

successful future application of these technologies will depend on their technical feasibility and ability to address the social, economic, and environmental challenges facing agriculture. Policies and regulatory frameworks will need to evolve in tandem with these technologies to ensure that their benefits are distributed equitably, particularly to the smallholder farmers who play a crucial role in global food production.

6.7 Conclusions

The integration of emerging wet-lab and dry-lab technologies into sugarcane breeding offers significant potential to address the many challenges faced by global agriculture, including rising demand for food, climate change, and the need for sustainable crop production. Wet-lab innovations such as CRISPR/Cas9 gene editing and nanotechnology have shown promise in improving sugarcane traits like disease resistance, yield, sugar content, and stress tolerance. These advancements are revolutionizing the way we approach genetic improvement and pest management. Similarly, dry-lab technologies, including multi-omics approaches and bioinformatics tools, enhance our understanding of sugarcane's complex polyploid genome and its responses to environmental stresses, allowing for more precise and targeted breeding strategies. However, applying these technologies raises significant ethical considerations that must be carefully addressed to ensure equitable benefits for all stakeholders, particularly smallholder farmers.

The challenges surrounding data ownership, transparency, access to technology, and environmental and health risks highlight the need for a thoughtful approach to the governance and implementation of these innovations. Ethical concerns must be integral to developing and deploying biotechnologies in sugarcane breeding, ensuring that the benefits are shared equitably while minimizing risks to biodiversity and traditional agricultural practices. These technologies' successful and ethical application relies on inclusive governance models, where farmers and other stakeholders actively engage in decision-making. This will foster trust, enhance collaboration, and ensure that technological advancements contribute to sustainable agricultural practices and food security in the long term. In summary, the future of sugarcane breeding is promising, with emerging technologies offering the potential to drive significant improvements in crop productivity, sustainability, and resilience. However, their successful integration into agricultural systems will require careful attention to ethical governance, transparency, and inclusivity, ensuring that all stakeholders can reap the benefits of these innovations while minimizing potential risks.

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Chapter 7
Sugarcane Breeding: Current Status and Breeding Priorities

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Abstract Sugarcane is a major crop cultivated globally for sugar production, especially in tropical and subtropical areas. Sugarcane breeding has evolved significantly, combining traditional hybridization with advanced biotechnological tools to improve yield, disease resistance, and stress tolerance. Current breeding programs focus on developing high-sucrose, high-biomass varieties with adaptability to diverse agro-climatic conditions. Key achievements include the development of disease-resistant cultivars and the incorporation of wild germplasm to enhance genetic diversity. Genomic selection, marker-assisted selection breeding, and the clustered regularly interspaced short palindromic repeats (CRISPR) techniques can potentially support the breeding process for developing better cultivars. Advanced production technologies that may be employed to enhance sugarcane efficiency-production and processing are limited due to economic, social, and regulatory constraints, especially in the regions of developing nations. Disease-free, climate adaptation, and better agronomic traits should be incorporated in the breeding programs for sustainable sugarcane in years to come. This chapter reviews the current status of sugarcane breeding, highlighting key developments in conventional

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hybridization, molecular marker-assisted selection, genomic breeding, and genetic engineering. It also explores the integration of wild germplasm to broaden genetic diversity and improve adaptive traits.

Keywords Conventional breeding · Crop improvement · Climatic resilience · Genetic diversity · Genomic selection · Hybridization · Genetic engineering · Sustainable sugarcane production

7.1 Introduction

Saccharum spp. is a tropical and subtropical crop globally (Verma et al. 2019a, b). Sugarcane is a highly efficient plant for capturing atmospheric sunlight and converting it into biomass, making it a valuable feedstock for ethanol production (Singh et al. 2019, 2020, 2022). However, the continuously rising demand to produce sugar and biofuels faces threats from pathogenic diseases, changes in climatic variables, and sustainable agriculture. Hence, there is a need to breed advance sugarcane varieties through breeding programs (Devi et al. 2024a, b; Kumar and Singh 2024a, b; Sharma et al. 2024). It is mainly grown for sugar and it supplies about 80% of the global sugar demand, while the remaining 20% comes from sugar beets (Verma et al. 2020a, b, 2024a).

Sugarcane is widely used to produce bioethanol, a renewable fuel. Bioethanol from sugarcane is more economically viable than that obtained from corn, as sugarcane can yield more per hectare and grow in tropical regions (Song et al. 2019; Malviya et al. 2020, 2022, 2024). Brazil has consistently used sugarcane ethanol to reduce their reliance on petroleum products. Apart from its uses in the energy and food industries, sugarcane plays numerous roles in carbon storage and land reclamation because it can absorb atmospheric CO_2 and enhance soil quality through biomass recycling (Chen et al. 2020; Huang et al. 2022; Li et al. 2024).

Sugarcane farming faces different challenges that negatively affect plant productivity and sustainability (Solanki et al. 2023; Kumari et al. 2022a, 2023). Sugarcane crop is prone to several diseases caused by fungi, viruses, bacteria, and nematodes. Sugarcane diseases, such as smut (*Sporisorium scitamineum*), mosaic virus, scald (*Xanthomonas albilineans*), and ratoon stunting disease (*Leifsonia xyli* subsp. *xyli*), are known to reduce crop yield. These diseases have become widespread because of the transport of agricultural products worldwide and changes in climatic conditions that determine where pathogens are found. Sugarcane smut, which initially affected only specific areas, shifted its geography to the globe because of the often-transferred infected plant material. It is a major challenge to breeding programs because new types of sugarcane not only produce high yields but also withstand a constantly changing range of diseases (Song et al. 2022; Sharma et al. 2021, 2022).

The global warming poses a significant threat to sugarcane production. Sugarcane relies heavily on tropical and subtropical climates for healthy growth (Verma et al. 2020c, d; Jagadesh et al. 2024), making it highly sensitive to changes in

environmental conditions such as air temperature, rainfall, drought, floods, and cyclones (Verma et al. 2020e, f; Anas et al. 2021a, b).For instance, the central south region of Brazil, a major sugarcane producer, is already experiencing the impacts of drought. The climate change upregulated the frequency of plant pathogenic diseases since most of them are known to develop within warm and humid environment (Malviya et al. 2021; Guo et al. 2022, 2023). The overuse of fertilizers and pesticides in farming, there is a loss of plant species and atmospheric pollution (Verma et al. 2021a, b; Joshi et al. 2022; Xu et al. 2022).

The modern breeding strategies are sought to produce promising, stable, and sustainable sugarcane varieties. In fact, because of multiple gene copies in the sugarcane polyploid genome, standard breeding can be lengthy and resource-consuming. The use of genomic selection and marker-assisted breeding has changed the paradigm of sugarcane breeding. Recent developments in clustered regularly interspaced short palindromic repeats (CRISPR) have a new way to breed sugarcane. CRISPR-Cas9 is a versatile gene editing technique that can directly add or change genes present in the sugarcane genome for desirable traits like disease resistance, stress tolerance, and higher biomass yield (Afghan et al. 2024). The ability to make precise modifications to the sugarcane genome can significantly speed up the development of new varieties, which is especially important for adapting to climate change (Singh et al. 2021, 2023; Verma et al. 2024e).

This chapter provides an overview and elaborates the current status and future breeding priorities from the aspects of advance strategies, with a special emphasis on sugarcane smart breeding. It accelerate the breeding process and technological applications, particularly in highly polyploid crop, i.e., sugarcane (Reddy et al. 2022; Kumari et al. 2022b; Ariyoshi et al. 2024).

7.2 Disease Resistance Breeding

With the increasing menace of diseases in sugarcane, disease-resistant breeding is essential for sugarcane production. In this way, scientists can develop varieties that have natural resistance to pathogens. Therefore, the need to use chemicals to treat crops is eliminated, thus improving the quality of crops produced.

7.2.1 Conventional and Marker-Assisted Breeding (MAB)

Traditionally, sugarcane breeding relies on cross-breeding plants to incorporate genes resistant to diseases from other varieties. However, this process is long and tedious, and it may take 10–15 years to develop a new variety (Lu et al. 2024). The ‘CO 86032’ variety in India, which has resistance to rust and smut, has been developed after several years of crossing through selection, showing that traditional methods lack the means for quick development to meet the challenges posed by

rapidly mutating pathogens (Viswanathan 2024). The last few years presented some significant improvements in genomics that have impacted the process of sugarcane breeding, especially using marker-assisted breeding (MAB). Marker-assisted breeding (MAB) allows breeders to identify and monitor genes associated with disease resistance in animals, facilitating the selection of resistant individuals for breeding programs (Cursi et al. 2022). This technology helps breeders pinpoint specific DNA markers linked to resistance, accelerating the process of developing disease-resistant breeds. The marker-assisted breeding success story includes RB92579—the 'Star' variety developed in Brazil. There is good resistance to this variety against several diseases, such as rust and smut, and therefore, its adaptation across South America is considered high due to the impact of disease resistance and high yield (Afghan et al. 2024).

7.2.2 Genomic Selection and CRISPR Technology

Genomic selection (GS) and CRISPR-Cas9 base gene-editing tools assist in identifying the significant development of variety from its genome, thereby helping in the rapid development of resistant varieties (Saini et al. 2023). On the other hand, CRISPR-Cas9 has the potential to directly target and modify genes associated with disease susceptibility. With the help of CRISPR, the resistance of sugarcane to leaf scald disease has been improved by modifying the genes responsible for the recognition of the infected tissue along with defense reactions (Kumar and Pathak 2019; Kumar et al. 2020; Kumar and Naik 2020).

7.3 Breeding Climate-Resilient Sugarcane Varieties

The era of climate change has increased and expanded the various diseases that affect the growth and development of sugarcanes. For example, increases in ambient air temperatures and changes in rainfall patterns have favored the development of pathogens in areas where they've never thrived before. In developing disease-resistant varieties, it is also essential to consider climate change (Linnenluecke et al. 2020). Awareness about cultivating climate-resistant and disease-resistant sugarcane varieties—such as 'LCP 85-384' developed in Louisiana to resist rust disease, mosaic virus, and water stress—is an important strategy for improving compact sugarcane yields. The mentioned varieties not only withstand local diseases but also are well adapted to the changing weather conditions in recent years (Blanchard et al. 2024). Breeding programs should focus on stresses like drought, heat, and flooding tolerance to help overcome the unfavorable climatic changes affecting sugarcane production and quality (Table 7.1).

Table 7.1 Development of resilient sugarcane varieties through breeding programs

Country	Variety	Institution	Key traits	Performance	Impact on production and sustainability
Brazil	RB867515	RIDESA	Drought tolerance, disease resistance (brown rust, mosaic virus), high sucrose content	High yield in low-rainfall regions	Reduces irrigation requirements and low fungicide use
India	Co 0238	ICAR-SBI	High water-use efficiency, high yield, resistance to red rot	Yields >100 tons/ha in drought-affected regions	Reduces water consumption, stabilizes yields in water-stressed areas
South Africa	N41	SARI	Heat and drought tolerance, frost resistance	12% higher yields in hot climates	Mitigates yield loss from heat stress, ensures reliable production
Australia	Q208	Sugar Research Australia	Flood tolerance, heat and pest resistance (grayback cane grub)	Maintain yield during flooding, heat waves	Reduces flood-related replanting costs, lowers pesticide use

(continued)

Table 7.1 (continued)

Country	Variety	Institution	Key traits	Performance	Impact on production and sustainability
Mauritius	M1176/77	MSIRI	Salt tolerance, moderate drought tolerance, high sucrose content	30% higher yield in saline soil	Expands cultivation in saline areas, reduces soil remediation costs
Thailand	KKU 99-02	Kasetsart University	Drought tolerance, high biomass yield	High yield in sub-tropical regions	Supports biofuel production, reduces irrigation dependency
Pakistan	CP 77-400	Pakistan Agricultural Research Council (PARC)	Drought and heat tolerance, red rot resistance	Well perform under low rainfall and high temperatures	Reduces irrigation, enhance resilience to red rot disease
China	ROC 22	Yunnan Agricultural University	Cold tolerance, high sucrose content, pest resistance	Sustain high yield in cold climates	Expand sugarcane production to temperate regions, reduces pesticide use
USA (Florida)	CPCL 95-1430	USDA-ARS	High water-use efficiency, high fiber content	Sustain yield in wet and dry seasons	Support biofuel production and efficient water use in variable climates
Egypt	G. T. 54-9	Sugar Crops Research Institute	Drought and salt tolerance, high sugar yield	Outperforms other varieties in saline soils	Expand cultivation in coastal regions, reduces irrigation costs

(continued)

Table 7.1 (continued)

Country	Variety	Institution	Key traits	Performance	Impact on production and sustainability
Colombia	CC 85-92	Cenicaña	High yield potential, drought and disease resistance	Consistently high yields in water-limited regions	Increases profitability for farmers, reduces losses from water scarcity
Mexico	Mex 69-290	Instituto Nacional de InvestigacionesForestales, Agrícolas y Pecuarias (INIFAP)	Drought tolerance, disease resistance, high sucrose content	High yield in arid regions with limited water supply	Less irrigation needs, reduces reliance on chemical inputs
The Philippines	Phil 99-1793	Philippine Sugar Research Institute	Flood and disease tolerance, high biomass	Thrives in waterlogged conditions	Ensure productivity in flood-prone areas, supports bioenergy production
Cuba	C89-147	Estación de Investigaciones de la Caña de Azúcar (INICA)	Resistance to drought and rust, high sucrose content	Performs well in dry and disease-prone environments	Reduces fungicide use and ensure stable production in semi-arid regions
Indonesia	PS 864	Indonesian Sugar Research Institute	Drought and flood resilience, pest resistance	Performs well under erratic rainfall	Reduces crop failures due to extreme weather, increases farmer income
Vietnam	VN84-413	Southern Institute of Agricultural Science and Technology	High yield, drought, and disease resistance	Thrives in regions with erratic monsoon patterns	Reduces water input and stabilizes yields during drought periods

(continued)

Table 7.1 (continued)

Country	Variety	Institution	Key traits	Performance	Impact on production and sustainability
Argentina	LCP 85-384	Instituto Nacional de TecnologíaAgropecuaria (INTA)	High fiber content, drought tolerance, frost resistance	Performs in cold and dry seasons	Suitable for biofuel production, resilient in extreme weather
Kenya	KEN 83-737	Kenya Agricultural and Livestock Research Organization (KALRO)	High drought and disease tolerance, pest resistance	High yield in semi-arid regions	Reduces water requirement, lowers pesticide and fungicide use
Nigeria	NCo 376	National Cereals Research Institute	Disease resistance (smut, rust), drought resilience	Thrives in regions with erratic rainfall	Limits crop loss from diseases, increases productivity in marginal areas
Fiji	Mana	Fiji Sugar Corporation	High sucrose, pest and disease resistance	High yield despite climate variability	Supports sugar export industry, reduces pest management costs

7.3.1 Drought, Heat, and Flood Resilient Varieties

Sugarcane is susceptible to drought stress because its growth requires sufficient water and nutrients. In this regard, the current developments in genomics breeding have helped to develop drought-tolerant sugarcane varieties. While breeding for drought resistance, growers have successfully selected plants with deeper root systems, enabling them to access water from deeper soil layers. They have also improved the plants' ability to assimilate carbon dioxide through photosynthesis under stress conditions (Aziz and Masmoudi 2025). For example, the 'RB867515' developed in Brazil shows improved drought tolerance and enhanced root systems able to tap deep water. This variety has thrived in the semi-arid regions of northeast Brazil, where other sugarcane varieties have been difficult to grow (Batista et al. 2024) (Table 7.1).

The another primary focus of developing sugarcane varieties that are 'heat tolerant' or can grow in warm conditions. Heat-tolerant varieties are produced through breeding for the ability to produce heat shock proteins (HSPs) to cope with high

temperature stress at cellular level. The 'Q208' sugarcane variety has proved promising and progressive research results in Australia in high temperature regions (Young 2018). Selection for the flood tolerance is more difficult because several genes associated in the resistance to soil flooding. Still, some studies have attempted to develop varieties that occasionally endure waterlogged conditions in recent years. For instance, the 'Co 0238' sugarcane variety, primarily grown in India, has shown moderate tolerance to soil flooding regimes accompanied by high sugar yields (Aley et al. 2022; Reddy et al. 2022; Dey et al. 2023c).

7.4 Climate-Resilient Sugarcane and Sustainable Agriculture

Advanced climate-stable sugarcane varieties have to be developed to overcome the challenges associated with unfavorable climatic factors (Verma et al. 2024a, c). Sustainable practices, such as precision agriculture, integrated water management, and soil conservation practices should be implemented so that sugarcane crop can be grown during unfavorable climatic situations. Ariel images obtained by using drones and remote sensing help farmers to easily monitor soil moisture content, plant health, and weather conditions. Such techniques enhance the sustainable use of water, fertilizer, and pesticides for crop production, minimizing climate change stress effect on sugarcane production (Table 7.1). Application of water conservation techniques like drip irrigation and rainwater harvesting help avoid excessive dryness. In dry areas, such techniques have been applied with significant impacts on yield after stress (Kumar et al. 2017, 2021b, 2022; Kumari et al. 2022a, b).

Soil and water management practices that include cover cropping, mulching, and conservation tillage contribute to increased soil structural stability, water holding capacity (WHC), and reduced crust formation and water erosion, enhancing sugarcane fields' resilience during flooding or drought condition. These approaches improve the sustainability of sugarcane farming, carbon stock, and soil health (Hidangmayum et al. 2023).

Climate change affects sugarcane production through high temperatures, irregular rainfall, and various natural disasters. Drought, heat, and flooding stress are the foremost and severe hazards that impact crop yield and quality (Headley et al. 2024). However, there is a prerequisite to breed climate-resistant varieties of this crop to guarantee the future sustainability of sugarcane production under adverse environmental conditions. The prospects for scientific progress in genomics and molecular selection and progress in sustainable agriculture open up further opportunities for developing climatic resilient sugarcane cultivars (Dey et al. 2023a; Sharma et al. 2023b, d).

7.5 Impact of Agro-Technological and Breeding Strategies on Sugarcane Production

Agro-technological strategies are essential for increasing the efficiency of sugarcane production, improving resource use efficiency, and ensuring sustainability under changing environmental constraints. These approaches, including irrigation, nutrient, pests, and precision agriculture strategies, are crucial for reducing the adverse effects of pathogenic diseases and climate changes (Verma et al. 2022, 2023). The application of agro-technological developments in combination with the ongoing breeding programs contributes to improving the plants' resistance characteristics and guarantees sustainable sugarcane production in the future (Table 7.1).

Genomic selection is an effective way for sugarcane varietal development. For example, a crop variety developed for drought conditions can achieve higher yields when combined with advanced applications. In the same way, development of disease-resistant varieties should be integrated with pest management to prevent pest buildup. Sugarcane breeding programs have incorporated modern irrigation techniques, resulting in water-use efficiency and disease/drought-resistant varieties (Khonghintaisong et al. 2024). It has also increased crop output and efficiency while making sugarcane sustainable production crop in the era of climate change (Sharma and Kumar 2023). Sugarcane breeding programs also ensure sustainable production by introducing advanced agro-technologies. Adopting efficient resources reduces chemical inputs, and breeding producer-tolerant varieties provides a sustainable solution for pathogen and climate change issues. Appropriate irrigation techniques, nutrients, and pest control are vital to enhance sugarcane productivity. These approaches minimize the effects of pathogenic pollution and climate change factors, and support breeding programs with improved sugarcane varieties. Advanced breeding methods and agro-tech innovations are better solutions to achieving sustainable production of this crop in years to come.

Different breeding strategies have recently been applied to improve the climatic adaptability of crops through breeding practices such as Hybridization, selection, and more complex processes such as marker-assisted selection (MAS) and genetic engineering (Karam et al. 2023; Kumari et al. 2023). Both approaches have their own advantages and disadvantages. Therefore, it is recommended to combine the key features of each method to implement a more comprehensive solution (Kumar et al. 2021c; Zhang et al. 2024).

7.5.1 Conventional Breeding Strategies

Traditional breeding strategies, comprising crossing and phenotypic selection for desirable traits, have been widely applied in sugarcane breeding programs to develop better genotypes with tolerance to climate change.

7.5.1.1 Hybridization and Phenotypic Selection

Biologically diverse sugarcane varieties are crossed to incorporate the favorable characteristics of the two parent genes. This approach has been widely applied to enhance the resistance of sugarcane varieties to environmental stresses. For instance, breeders can combine a variety with good drought resistance with another high sugar content to develop drought-resistant varieties with potential yield productivity. Brazil developed RB867515 variety by combining sugarcanes with drought tolerance and high yield potential. This variety has been widely cultivated in semi-arid regions where traditional varieties are not cultivated due to insufficient water supply. This is evident since hybridization enables breeders to stack up several desirable genes to a particular variety with abiotic and biotic stress responses enhanced through heat, drought, and pathogen resistance. Cross-breeding takes several generations to establish the desired traits from a plant and, hence, hybridization is time-consuming. Besides, the results are sometimes unpredictable because multiple genes control most of the polygenic traits.

This strategy involves selecting a pair of plants for breeding from a population based on their characteristics. For instance, sugarcane breeders have developed varieties through phenotypic selection with high temperatures and irregular rainfall in Maharashtra, India, which has increased productivity during heat stress condition (Aggarwal et al. 2024). Phenotypic selection can easily be implemented by farmers and breeders with limited resources. This technique is also effective for a wide range of phenotypic traits, including plant height, tillering ability, and sugar content. Various other interactions make phenotypic characterization as a complex issue; for example, genes and environmental factors significantly contribute to phenotypic variations (Jamalluddin et al. 2021; Khew et al. 2022; Wang et al. 2024).

7.5.1.2 Use of Genetic Markers and Gene Introgression

Marker-assisted selection (MAS) involves developing molecular markers, such as Single Nucleotide Polymorphisms (SNPs), that are genetically linked to specific traits of interest. Once these markers are identified, they can be employed to select sugarcane populations with the required genes. For instance, Australian researchers have applied this technique to develop improved sugarcane varieties that showed enhanced heat tolerance efficiency, which is achieved through marker-assisted selection of the genes for heat shock proteins (Yao et al. 2025). Use of MAS shortens the breeding cycle, as plants with the required characteristics are selected at an early stage even if they have not fully matured. It also provides high accuracy to the breeding exercise due to its ability to select individual genes to enhance climate resilience (Singh et al. 2015; Shahzad et al. 2022; Wilson and VanBuren 2022). MAS is more beneficial for incorporating the genes of interest from wild relatives or other crops into sugarcane cultivars. Drought tolerance genes from *Saccharum spontaneum* can be directly transferred to commercial varieties through MAS,

developing new sugarcane varieties with high resistance to water stress (Srinivasa Rao et al. 2016; Henry 2020; Ariyoshi et al. 2024; Mangal et al. 2024).

7.6 Genetic Engineering: Introducing Novel Traits for Climate Resilience

Genetic engineering approaches are utilized to introduce new genes into sugarcane varieties. This technique allows scientists to transfer genes from other organisms or even modify existing genes within sugarcane to enhance desirable traits (Kumar et al. 2024). It can directly change the plant's DNA without conventional breeding.

7.6.1 CRISPR-Cas9 and Transgenic Approaches

CRISPR-Cas9, a modern effective tool, can cut any specific gene of DNA and is utilized to modify genes that control stress tolerance to biotic factors. Brazilian scientists applied CRISPR to edit genes involved in stomatal opening in sugarcane, improving water-use efficiency (Yao et al. 2025). These edited plants showed enhanced drought tolerance capacity with no negative impact on sugar yield, so it is recommended for proper development in insufficient water available areas. It is also faster than other breeding techniques because it avoids several generations of selection and recurrent cycles of backcross breeding. Another drawback of genetic engineering is the problem of coming up with policies on GMOs and the general acceptance of GMOs in the market. Further, genetic engineering is expensive and practicable only by those with specialized equipment and resources, making it difficult for small breeding entities (Batista et al. 2024).

Besides CRISPR, transgenic methods have been employed in previous years to transfer genes from other species to sugarcane. The desirable genes have been moved from *Arabidopsis*, a drought-resistant plant into sugarcane to increase its drought tolerance efficiency. It proved promising to enhance sugarcane resistance to drought and heat stress conditions. South African scientists used a gene from *Arabidopsis* (*AtDREB1A*) that helped plants to survive during drought conditions by better regulating the water use efficiency (Ravikumar et al. 2014). The developed transgenic sugarcane plants overexpressing the *HbDREB* gene exhibited enhanced stress tolerance under water stress, suggesting the application of genetic engineering techniques for improving climate change adaptation.

7.6.2 *Integrated Breeding Strategies for Climate-Resilient Sugarcane*

The significance and drawback of each method of breeding, using the identified method of breeding, sugarcane climate resilient varieties can be achieved by combining conventional breeding approaches. Nowadays, sugarcane breeders in Brazil are using different breeding techniques to develop new sugarcane varieties that are resistant to new pests, diseases, and stresses. The production of climate-tolerant sugarcane varieties are the way of global sugar/bioenergy production on the impact of climate change (Saini et al. 2023; Lu et al. 2024). It is believed that the best breeding strategies would require both conventional and molecular breeding to produce sugarcane varieties that are drought and heat-resistant, as well as other environmentally stress-resistant species.

7.7 Collaborative Research and Development

7.7.1 *International Collaborations*

- *Shared Knowledge and Resources:* Due to the nature of sugarcane and its massive impact on the international market, there must be interaction. The developed nations have established expertise and resources in sugarcane breeding and cross-breeding programs, making them well suited to share their knowledge and expertise with others.
- *Cross-Institutional Programs:* Close cooperation with research institutes, universities, and private companies will help enhance the work progress by developing new resistance sugarcane varieties. These include the International Consortium for Sugarcane Biotechnology (ICSB), where genetic material, data, and other breeding information can be shared for better varietal development.

7.7.2 *Public-Private Partnerships (PPP)*

- *Resource Sharing:* Depending on the association of breeding programs, it requires vast amounts of research fund, which can only be provided through PPPs model. With assistance from private companies, governments can fund long-term infrastructure, research, and technology transfer to scale up breeding ventures for sustainable sugarcane.
- *Adoption and Commercialization:* LPO participation can also assist in developing new varieties with commercial purposes quickly, helping farmers adopt climate and disease-resistant varieties on large-scale cultivation.

7.7.3 Focus on Sustainability

- *Integrating Sustainability in Breeding:* Next-generation breeding programs have to stick to the environmental concerns, whereby the objective of sugarcane production has a limited impact on the environment. Growing varieties that need less water, fertilizers, and pesticides is also possible, which will help in the advancement of sustainable agriculture.
- *Biodiversity Conservation:* The essential factor that breeding programs should consider the issue of species diversity, which involves the local varieties that can be very beneficial as they contain the gene pool for some essential traits. Maintenance of the perceived sugarcane genetic variation is critical for meeting future breeding requirements.

7.7.4 Climate-Adaptation Strategies

- *Regional Adaptation:* Future breeding programs should take into consideration some challenges that are unique to the region. For instance, in the areas that experience increased sea levels, there will be a need to grow varieties that are resistant to salty soil, while in the dry areas, the most critical varieties will be those that are drought-resistant.
- *Real-Time Data Utilization:* Climate data collected in real time and climate modeling help breeders make decisions about developing appropriate varieties for future climatic conditions. Such an approach may lead to the development of subtypes that are not only resistant to the present climate conditions but also those forecasted to occur in the future.

7.8 Conclusions, Challenges, and Future Prospects on Sugarcane Breeding

Sugarcane breeding has made significant progress over the years, leveraging both conventional and modern biotechnological approaches to develop high-yielding, disease-resistant, and stress-tolerant varieties. Despite these advancements, challenges such as climate change, pest and disease evolution, and the demand for higher sucrose content and biomass productivity persist. By integrating multidisciplinary approaches, sugarcane breeding can meet global demands for sugar, bioenergy, and other value-added products while ensuring sustainability and resilience in the face of changing agricultural landscapes.

Sugarcane is a polyploidy, genetically more complicated than other species. The multiple copies of each gene make selecting desirable traits, such as disease resistance, climate resilience challenges. A relatively large and complicated sugarcane

genome complicates genome sequencing and markers development, thereby hampering the usage of modern tools, such as genomic selection and CRISPR. Sugarcane has long growing periods, which takes 10–12 years from cross-breeding to developing varieties for commercialization. The new and adaptable varieties take longer, providing farmers a much slower supply. Sugarcane that has been developed to grow in a variety of settings, any breeding process obviously covers multiple settings, and it naturally inflates the time taken for a specific breed to be tested under different conditions. Some of the major diseases, i.e., red rot, smuts, and sugarcane mosaic virus, are recurrent and cause degradation of resistance in varieties. To achieve this, there must be a continuous development of new resistance genes. Breeding for long-lasting resistance is a challenging and important task. Breeding for long-lasting resistance is a significant task. Substantial numbers of strains can show resistance to a particular pathogen in a short duration, but changes in the pathogen population dynamics affect this resistance in the long duration.

- *Selection Efficiency:* Genomic selection that employs DNA markers to estimate phenotypic values of progeny before being tested for performance in the actual field is becoming popular. This approach may also help to speed up the identification of a desirable characteristic to stress resistance and enhance the selection's efficiency.
- *Data-Driven Breeding:* By leveraging large-scale genomics and upcoming machine learning tools, the accuracy of genomic selection can be improved, improving breeders' chances of identifying resilient traits.
- *Precise Gene Targeting*: The advance and powerful CRISPR-Cas9 technology suitable for sugarcane breeding involves modifying or regulating climate-resilient genes and genes that resist diseases. For instance, genes regulate drought tolerance in sugarcane, and their efficiency in arid regions is promoted via gene editing.
- *Pathogen Resistance:* CRISPR is effective in introducing or modifying genes with desirable characteristics, including resistance to emerging pathogens. For example, the *SRL1* gene associated with resistance to red rot might be further developed to enhance effectiveness.
- *Regulatory Challenges:* The advancement of CRISPR technology remains, with some challenges related to regulating genetically edited crops, especially in certain countries. Some of these challenges will be vital to scaling up CRISPR in sugarcane breeding programs or for CRISPR to become mainstream.
- *Accelerating Trait*: Marker-assisted selection (MAS) is particularly useful for sugarcane breeding because of its complex genome. MAS helps breeders identify and select for specific genes associated with desirable traits, like disease resistance or yield, even in a crop with a large and complex genome.
- *Stacking Traits*: Through MAS, desirable traits can be combined in a single sugarcane variety, producing crops immune to biotic stresses.
- *Transgenic Approaches:* Another area of development regarding stress tolerance possibility of transgenic approaches to transfer genes to other species. For instance, a transgenic procedure could transfer genes responsible for drought

compatibility among specific grasses into sugarcane to make it capable of tolerating minimal rainfall.

- *Epigenetic Modifications:* Research may be done in the future regarding the effects of epigenetic modifications on increasing the expression of climate-related traits without changing the DNA sequence. These fields are attracting a lot of interest due to their potential to revolutionize various areas of significant scientific developments.

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Chapter 8
Exploring Microbial Roles in Sugarcane Breeding: From Soil Health to Plant Growth Promotion

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Abstract The integration of beneficial microorganisms in sugarcane breeding holds promising potential to enhance crop productivity, resilience, and sustainability. This chapter delves into the diverse roles of microorganisms, from soil health improvement to plant growth promotion, within sugarcane cultivation. Microbial communities in the rhizosphere, including bacteria, fungi, and actinomycetes, influence nutrient cycling, soil structure, and plant health. Specific microbial taxa, such as nitrogen-fixing bacteria, phosphate-solubilizing organisms, and plant growth-promoting rhizobacteria (PGPR), improve nutrient accessibility, root development, and stress tolerance. By exploring the symbiotic and associative interactions between sugarcane and these microorganisms, this chapter provides insights into harnessing microbial diversity for breeding strategies aimed at improved yield and resilience. Additionally, advancements in microbial biotechnology, such as biofertilizers and biostimulants, are discussed as essential tools in sustainable sugarcane agriculture. Emphasizing the ecological and economic advantages of these microbial interventions, this chapter presents a framework for incorporating microbial insights into breeding programs that support sustainable, high-yielding sugarcane production.

Keywords PGPRs · Microbiome · Sugarcane breeding · Biofertilzers · Sustainable production

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8.1 Introduction

Grown in almost 110 countries worldwide, sugarcane is a significant crop. Although the word "sugarcane" refers to a group of *Saccharum* species, it is most frequently used to refer to interspecific hybrids of *Saccharum* spp. and farmed *Saccharum officinarum* L. Since sugarcane serves as a raw resource for a variety of sectors, including food and fuel, it has evolved into a multipurpose agro-industrial crop over time (Fischer et al. 2012). Due to soil degradation and the accumulation of pests and diseases, sugarcane is mostly produced as a monoculture for extended periods of time, which results in a loss in production (Malviya et al. 2022).

The rhizosphere is abundant in microbial variety and numbers. These microbe communities, which have various functions, consist of endophytes and plant growth-promoting rhizobacteria. Bacteria make up the majority of the microorganisms in the rhizosphere, occupying as much as of the entire root surface (Van Loon 2007). Approximately 2–5% of these rhizobacteria are identified as plant growth promoters. Numerous plant growth-promoting rhizobacteria (PGPRs) can fix nitrogen, solubilize inorganic substances like phosphate, produce plant hormones/regulators and siderophores, and generate compounds that help manage phytopathogens. Therefore, PGPRs and other bacteria in the rhizosphere are increasingly recognized as vital components of sustainable agricultural systems. The most prevalent bacterial genera found in the rhizosphere include *Rhizobia, Bacillus, Arthrobacter, Agrobacterium, Pseudomonas, Azotobacter, Micrococcus, Alcaligenes, Mycobacterium, Cellulomonas*, and *Flavobacter* (Teixeira et al. 2010; Prashar et al. 2014).

Chemical fertilization techniques disregard the biological capacity of the rhizosphere or roots via enhancing the mobilization and uptake of nutrients and reducing interactions between plants and microorganisms in the rhizosphere, despite the fact that their use in crop production increases yields by an average of about 50% when compared to production without their use (Meena et al. 2017). Several reports confirmed that microorganisms that support plant growth may enhance plant nutrition and decrease the demand for pesticides (Aloo et al. 2019). Since the industrialization of agriculture, which was prompted by the need to increase the amount of food available to the general public, has resulted in serious environmental and social problems, plant growth-promoting rhizobacteria are a great option for farmers facing the new challenges of modern agriculture. Maintaining high output with little environmental effect is imperative (Pérez-Montaño et al. 2014).

Microorganisms are essential in supporting plant growth and health throughout their entire life span (Vandenkoornhuyse et al. 2015). Consequently, it is crucial to determine all factors and sources that influence the plant microbiota. For many years, researchers have examined how plant genotype and soil quality affect the rhizosphere microbiome's diversity (Lundberg et al. 2012). However, the impact of breeding on the microbiome has only been recognized recently. Another critical finding pertains to the seed microbiome; microorganisms associated with plants, including beneficial microbes, passed down through seeds to subsequent

generations. Notably, microbial communities that are specific to genotypes are even more distinct in seeds (Chen et al. 2020). Overall, understanding how core microbiomes are transferred between generations (Berg and Raaijmakers 2018), which can be subjective by selective breeding (Mendes et al. 2019), presents opportunities for pioneering approaches in plant breeding and protection strategies (Cordovez et al. 2019; Kusstatscher et al. 2021).

Worldwide, sugarcane breeding initiatives are creating new cultivars that yield higher amounts and resist key diseases to enhance the profitability and sustainability of the sugar-energy sectors they support. On Reunion Island, the efforts for genetic improvement of sugarcane started in 1929. Ongoing breeding initiatives have been implemented to develop varietal resistances aimed at managing significant diseases, and these efforts continue today. Due to the extreme agroclimatic variance among different production regions within the industry, it was essential to progressively establish a network of seven decentralized breeding programs to facilitate genetic advancement throughout the industry (Dumont et al. 2022). Climate change has caused numerous disruptions in the environment, including extreme temperatures, drought, waterlogging, and altered weather patterns, all of which negatively impact global crop production. Various environmental and human-induced factors exert abiotic stress on plants, including temperature, salinity, drought, heavy metals, fluctuating temperatures, unpredictable rainfall, and increasing instances of drought, contributing to changes in agricultural cycles (Verma et al. 2019a, b, 2023, 2024a,b). Over 50% of farming losses result from heat, salinity, drought, and heavy metal stresses, affecting the quality and quantity of produce (Salam et al. 2023). Recently, insights into the molecular mechanisms by which PGPR helps plants tolerate different stress factors have emerged. Concurrently, integrating recent progress in plant-microbiome interactions with innovative omic technologies is expected to pave the way for a comprehensive method to "prime" plants for adverse conditions (Mellidou and Karamanoli 2022).

This chapter explores the sugarcane microbiome and its role in sugarcane breeding mechanisms through which these microorganisms impact sugarcane health, including hormone modulation, pathogen resistance, and abiotic stress mitigation.

8.2 Plant Growth-Promoting Microbes (PGPMs)

In addition to producing phytohormones, including indole-3 acetic acid (IAA), jasmonic acid (JA), ethylene (ET), cytokinins (CKs), and gibberellic acid (GA). PGPMs can increase the accessibility of particular nutrients, such as phosphate solubilization and nitrogen (N2) fixation. PGPR can use both direct and indirect methods to encourage the increase in the host plant it is invading. Direct routes include increased iron absorption, phosphate solubilization, and plant hormone synthesis. Production of antibiotics, competition for resources, parasitism, suppression of pathogen toxins, and activation of resistance mechanisms are examples of indirect advantages (Elnahal et al. 2022). How plants protect themselves from abiotic

stresses such as drought and salinity explained by the function of PGPR in producing phytohormones and related signaling molecules (Jochum et al. 2019).

According to Abbas et al. (2019), PGPR may potentially alter the shape of roots, boosting their surface area, and improving their functionality. Furthermore, by quickly colonizing and obtaining a better provider of nutrients, PGPR can prevent the growth of other microorganisms (El-Saadony et al. 2023). PGPR employs a number of techniques that can enhance both crop protection and plant health in general.

8.2.1 Nitrogen Fixation

Different PGP bacteria are capable of converting nitrogen through a process known as biological nitrogen fixation (BNF), which transforms atmospheric N2 into NH3. These bacteria, referred to as diazotrophs, possess the nitrogenase enzyme complex that facilitates the conversion of N2 gas into ammonia (Santi et al. 2013). Nitrogen-fixing bacteria convert inert nitrogen from the atmosphere into organic forms (Bakulin et al. 2007). As microbial inoculants, often, organisms that fix nitrogen are employed. The majority of nitrogen-fixing bacteria are found alongside plants that are leguminous. These bacteria can be categorized into free-living types (such as *Azotobacter* and *Azospirillum*), as well as symbiotic organisms like *Azolla, Rhizobium*, and *Frankia* (Gupta 2004). On the other hand, numerous bacteria have also been linked to non-leguminous plants (Mohammadi and Sohrabi 2012).

Numerous types of nitrogen-fixing bacteria have been found to live in non-leguminous crops sugarcane, wheat, rice, and maize. These bacteria successfully colonize multiple sections of the plant tissues. The relationship between nitrogen-fixing bacteria and their host plants has been shown to enhance plant growth and yield. Several nitrogen-fixing bacteria have been isolated from sugarcane, including *Burkholderia vietnamensis* (Govindarajan et al. 2008), *Stenotrophomonas* maltophilia and *Agrobacterium tumefaciens* (Xing et al. 2015), Novosphingobiumsediminicola and Ochrobactrum intermedium (Muangthong et al. 2015), *Pseudomonas* spp. (Li et al. 2017), and *Burkholderia anthina* (Malviya et al. 2019). Using 16S rRNA gene sequencing, 64 nifH gene-positive bacteria from sugarcane intercropped with legumes were found. Each bacterium had distinct plant growth-promoting capabilities, and all diazotrophs were categorized into diverse taxonomic groups (Solanki et al. 2018). BNF supports sustainable farming methods by acting as a substitute for nitrogen fertilizers. Numerous PGP bacteria have the ability to fix nitrogen from the atmosphere and transfer it to their host plants (Malviya et al. 2020).

8.2.2 Phosphate Solubilization

Phosphorus (P) is a crucial macronutrient that is integral to plant development and is involved in numerous metabolic processes. It is an essential component of life, found in various biological molecules, such as coenzymes, phosphoproteins, and phospholipids. Furthermore, P is a primary limiting factor for biomass production in terrestrial ecosystems. It contributes to the ongoing eutrophication of inland and coastal waters due to the widespread use of P fertilizers. The P cycle operates within specific ecosystems, including soil, streams, forests, and marine environments, and is closely linked to significant security concerns regarding the surrounding environment and human society. Phosphate solubilizing microorganisms (PSMs) represent a significant group of microbes that facilitate the availability of phosphorus in the soil, playing an essential role in the phosphorus cycle by transforming organic phosphorus, solubilizing inorganic phosphorus compounds, and accumulating substantial amounts of phosphorus in their biomass.

By producing phosphatase enzymes and organic acids, lowering soil pH, and enhancing chelation processes with additional sites for phosphorus adsorption, PSMs can convert soil phosphorus into soluble forms accessible to plants as orthophosphate (Tian et al. 2021). Many microbial organisms, such as bacteria, fungi, actinomycetes, and algae, can solubilize and mineralize phosphorus. Soil bacteria known to mobilize inaccessible phosphorus through solubilization and mineralization include Pseudomonas spp., Agrobacterium spp., and Bacillus circulans. Other bacteria that have been identified as phosphorus solubilizers and mineralizers consist of a variety of strains of *Azotobacter* (Kumar et al. 2014), *Bacillus* (Jahan et al. 2013; David et al. 2014), *Enterobacter*, *Erwinia* (Chakraborty et al. 2009), *Burkholderia* (Zhao et al. 2014; Istina et al. 2015), along with Kushneria (Zhu et al. 2011), *Ralstonia*, *Rhizobium* (Tajini et al. 2012), *Paenibacillus* (Fernández Bidondo et al. 2011), *Rhodococcus*, *Sinomonas*, *Serratia*, *Salmonella*, *Bradyrhizobium*, and *Thiobacillus* (David et al. 2014).

8.2.3 Biocontrol Agents

The diverse group of bacteria, plant growth-promoting rhizobacteria (PGPR) can influence a plant by either causing systemic resistance to pathogens or by causing local antagonism toward soil-borne pathogens. The compounds that antagonistic rhizobacteria create, such as siderophores and antibiotics, have been connected to indirect growth augmentation and disease control in a range of plants. When the pathogen and the inducing bacterium stay spatially apart, the induced systemic resistance (ISR) seen in plants is alike to the pathogen-triggered systemic acquired resistance (SAR). Rhizobacteria may need the plant's perception of ethylene and jasmonic acid for ISR, or they may use the salicylic acid-dependent SAR pathway to promote resistance.

8.2.4 Induced Systemic Resistance (ISR)

Induced systemic resistance (ISR) represents an active condition of defense triggered by an inducing factor following pathogen attack. Beneficial rhizobacteria can activate ISR, while pathogen-triggered resistance is known as systemic acquired resistance (SAR). This induced resistance offers broad-spectrum, non-specific protection against various threats, encompassing fungi, bacteria, viruses, insects, and nematodes (Beneduzi et al. 2012). SAR is characterized by the involvement of salicylic acid (SA) and modifications in gene expression associated with pathogenesis-related proteins. Most plant growth-promoting rhizobacteria (PGPR) utilize an SA-independent pathway to stimulate ISR, which comprises jasmonate and ethylene signaling. These hormones play a role in triggering specific sets of defense-related gene expression in plants and/or disseminating defense mechanisms throughout the plant tissues, resulting in host physiological and metabolic changes, that is, enhanced cell wall fortification, increased levels of proteins or defense-related enzymes (including glucanase, chitinase, etc.), as well as the production of phenolic compounds and phytoalexins (Pokhare et al. 2012).

The gathered evidence suggests that numerous characteristics of PGPR also play a role in triggering ISR. For instance, volatile organic compounds such as 2R,3R-butanediol released by *Bacillus subtilis*, *Pseudomonas putida*, and *Serratia plymuthica*. The effectiveness of PGPR strains in inducing systemic resistance differs among them, and the stimulation of systemic resistance depends on the specific traits of each strain. Combinations of compatible dual PGPR strains, which induce systemic resistance against various pathogens in multiple plant hosts, resulted in more effective disease suppression compared to the application of individual strains (Wang et al. 2021a, b).

8.2.5 Production of Phytohormones

Key microorganisms known as plant growth-promoting rhizobacteria (PGPR) have the ability to cause the production of phytohormones that are important for plant development, including auxins, gibberellic acid, abscisic acid, cytokinins, and ethylene. The advantages of incorporating PGPR into the soil include enhanced germination rates, improved root development, increased yield, better crop quality, expanded leaf area, higher chlorophyll levels, more significant nitrogen and protein content, enhanced tolerance to environmental stresses, increased shoot and root weight, prolonged leaf longevity, and improved resistance to pests and diseases (Stefen et al. 2022). Phytohormones control various cellular processes in higher plants and are crucial for coordinating multiple signal transduction pathways during the plant's reaction to abiotic stresses (Pieterse et al. 2009). Their vital function is facilitating adaptation to fluctuating environments from end to end growth and differentiation, well-established source/sink ratio modifications, and nutrient distribution (Wani et al. 2016).

Abiotic stresses trigger signal transduction pathways associated with the fundamental pathways through which plant hormones operate. Naturally occurring chemical substances called phytohormones are crucial for controlling plant development and yield. They also significantly improve plants' resistance to a range of biotic and abiotic stressors (Verma et al. 2024). The points of convergence among hormone signal transduction pathways are referred to as cross talk, which is essential for plant development and responses to both biotic and abiotic stresses. Hormones interact with plant growth-promoting rhizobacteria (PGPR), which form close associations with the roots of higher plants and also influence the levels of endogenous phytohormones, presenting a new framework for understanding hormonal interactions. The hormone ratios shift as the plant develops, and the specific balance between growth-promoting and growth-inhibiting hormones determines how plants respond. Additionally, the responsiveness of plant tissue to each hormone varies depending on stress exposure.

8.2.6 Stress Tolerance Enhancement

Microorganisms significantly contribute to agricultural production due to their unique characteristics that facilitate plant growth, as well as their ability to colonize plant tissues and form biofilms. Diverse abiotic stresses, including drought, salinity, and high temperatures, respond to these challenges within plant tissues (Grover et al. 2011). Plant growth-promoting (PGP) microorganisms or microbial inoculants help stabilize soil composition, prevent erosion, and mitigate plant diseases while also being capable of fixing atmospheric nitrogen, solubilizing mineral phosphates, decomposing organic waste, enhancing nutrient cycling, producing bioactive compounds, phytohormones, and siderophores, in addition to maintaining ionic balance, and influencing other physiological factors within plants (Talaat and Shawky 2017).

By producing exopolysaccharides (EPS), phytohormones, 1-aminocyclopropane-1-carboxylate (ACC) deaminase, and volatile chemicals; encouraging the accumulation of osmolytes, antioxidants; and controlling stress-response genes, beneficial microbes that colonize plant tissues improve drought resistance (Vurukonda et al. 2016). Previous research has shown that the drought tolerance of plants can be improved by various types of microorganisms, including fungi (Singh et al. 2011) and endophytic and rhizospheric bacteria (Dimkpa et al. 2009). During periods of drought, it has been observed that PGP bacteria potentially participate in nutrient cycling activities, such as phosphorus solubilization, nitrogen fixation, and siderophore production (Kim et al. 2012); additionally, they have been shown to enhance photosynthesis, promote root development, and reduce volatile stress emissions.

8.3 Microbiomes Associated to Sugarcane and How They Affect Plant Growth

Plant-associated microbiomes have a great deal of promise to improve plant health and increase agricultural system productivity. Beneficial plant microorganisms and their byproducts can be used in a contemporary biotechnology strategy to boost agricultural yields, boost nutrient availability, improve disease resistance, and fortify plants' tolerance to stress (Trivedi et al. 2017). The sugarcane plant forms connections by a range of microbes as it matures. Utilizing beneficial bacteria and fungus is a crucial tactic for encouraging plant growth and a viable means of satisfying the present needs for increased sustainability and production. Throughout the years, numerous reports have shown the presence of microbial communities in various sections of sugarcane and their contribution to promoting plant growth (Table 8.1).

8.4 Microbial Consortia and Their Synergistic Effects

Plant-associated microorganisms are essential to agricultural productivity. Even though several studies have confirmed that individual bacteria can help plants, it is becoming more and more clear that additional or synergistic effects may be anticipated when a microbial consortium—two or more interacting microorganisms—is involved. In an ecosystem, such as the rhizosphere, a variety of species can perform a variety of tasks, which contributes to this phenomenon. Thus, a variety of microbial actors in the rhizosphere, including fungi and PGPB, activate plant growth in a positive way through mechanisms like improved nutrient availability, phytohormone modulation, biocontrol, and resistance to biotic and abiotic stress (Santoyo et al. 2021). Bacterial consortia have been shown in numerous studies to improve plant development in both greenhouse and field settings. For instance, Jha and Saraf (2012) discovered that applying a multispecies consortium comprising *Micrococcus* sp., *Acinetobacter calcoaceticus, Brevibacillus brevis,* and *Bacillus licheniformis* increased *Jatropha curcas* plant yields.

This consortium can produce indole-3-acetic acid (IAA), 1-aminocyclopropane-1-carboxylate (ACC) deaminase, siderophores, and solubilize inorganic phosphorus. Similarly, in tomato, brinjal, and chilli seedlings, a bacterial consortium of *Azospirillum*, *Pseudomonas striata*, *Pseudomonas* sp., and *Leuconostoc mesenteroides* produced higher plant height and biomass (Jayashree et al. 2017). A group of drought-resistant bacteria (*Sphingomonas* sp., *Pseudomonas putida*, *Acinetobacter* sp., and *Azospirillum brasilense*) were shown to be able to adhere to seeds and colonize the rhizosphere of maize plants in a diverse study (Molina-Romero et al. 2017; Santoyo et al. 2021).

Table 8.1 Microbes associated with sugarcane and their role in Plant growth-promoting activity

Microbes	PGP activity	Reference
Novosphingobium sediminicola and *Ochrobactrum intermedium*	Nitrogen fixation	Muangthong et al. (2015)
Burkholderia	Plant-growth	Lonhienne et al. (2016)
Enterobacteriaceae, *Klebsiella*, *Enterobacter*, *Pantoea*	Growth-promoting	Rodrigues et al. (2016)
Actinobacteria	Nitrogenase activity	Wang et al. (2017)
Diazotrophs and Actinomycetes	Nitrogen fixation	Kruasuwan et al. (2016)
Diazotrophic	Cultivation influenced	Solanki et al. (2017)
Klebsiella pneumoniae VRE36	Nitrogen fixation	Bhardwaj et al. (2017)
Pseudomonas spp.	Nitrogen fixation and PGP	Li et al. (2017)
Enterobacterludwigii, *Bacillus amyloliquefasciens*, *Bacillus subtilis*, and *Enterobacteroryzae*	Growth-promoting	Tam and Diep (2017)
Bacillus spp.	Growth-promoting	Chandra et al. (2018)
Asticcacaulis, Burkholderia, Chitinophaga, Ensifer, Lysobacter, Pedobacter, Rhizobium	Growth-promoting	Armanhi et al. (2018)
Burkholderia, *Sphingobium*, *Rhizobium*, and *Enterobacter* genera	Plant growth and biocontrol	Rodrigues et al. (2018)
Burkholderia anthina	Growth-promoting	Malviya et al. (2019)
Herbaspirillum frinsingense and *Pantoea dispersa*	Nitrogen fixation	Da Silveira et al. (2019)
Streptomyces	Growth promotion	Wang et al. (2019)
Gluconacetobacter diazotrophicus, *Herbaspirillum rubrisubalbicans*, *Nitrospirillum amazonense*, and *Paraburkholderia tropica*;	Nitrogen fixation	Santosa et al. (2019)
Bacillus megaterium and *Bacillus mycoides*	Nitrogen-fixing	Singh et al. (2020)
Kosakoniaradicin citans and *Stenotrophomona smaltophilia*	Improve growth and stress-related gene expression	Singh et al. (2020)
Bacillus	Enhancing plant growth	Wang et al. (2020)
Streptomyces spp.	Biocontrol potential of Actinobacteria	Wang et al. (2021)

(continued)

Table 8.1 (continued)

Microbes	PGP activity	Reference
Endophytic and rhizospheric	Growth-promoting	Ferrara et al. (2011)
Methylobacterium	Growth promotion	Jirakkakul et al. (2023)
Bacillus wiedmanii and *Bacillus zhangzhouensis*	Growth-promoting	Bezerra et al. (2023)
Trichoderma asperellum, *Bacillus subtilis*, *Chaetomium globosum* (Cg6)	Biocontrol	Yamunarani et al. (2023)
Rhizobacteria	Growth-promoting	Gupta et al. (2024)

8.5 Microbiome Engineering for Sugarcane Breeding

Soil serves as a reservoir of microbial diversity from which plants actively select their microbiome to meet specific needs. In addition to soil, seeds, which carry the genetic information of plants during successive generations, also host various microbiota that provide essential microbial inoculum for agricultural practices. Essentially, plants are surrounded and inhabited by a wide array of microbiota, both externally and internally. The plant's genome and the genes of the associated microbiota in various plant tissues comprise the "plant microbiome," which together forms the holobiome, now recognized as a unit of selection known as the "holobiont." The "plant microbiome" not only supports the health of plants but also introduces significant genetic diversity that has yet to be utilized in traditional breeding methods, where plant breeders have primarily relied on the genetic variability of the host to create high-yielding, disease-resistant, or drought-tolerant varieties. This new understanding of the microbiome, especially in the rhizosphere, which contributes genetic diversity to plants, heralds new possibilities for breeding aiming at the development of next-generation crops that are less dependent on synthetic inputs and more resistant to pests, diseases, and environmental fluctuations (Gopal and Gupta 2016).

A single strain identified through controlled inoculation trials or plant growth-promoting (PGP) in vitro screening tests activities often makes up conventional inoculants employed in modern agricultural techniques. Despite being widely used, these approaches fall short in capturing crucial elements of interactions between microbes and plants. According to earlier research, the plant microbiome is made up of a very complex and varied community that is frequently maintained by several interactions between microorganisms and their hosts. Furthermore, the positive impacts of the microbiota are often attributed to the synergistic interactions among microorganisms (Niu et al. 2017; Correa de Souza et al. 2020).

These days, several cutting-edge technologies are being employed to examine the interactions between microbes and plants, as well as their roles in various features and routes. The sugarcane microbiome and its interactions with plants are analyzed using high-throughput sequencing, metagenomics, and metatranscriptomics. Sugarcane genes or bacteria can be engineered using CRISPR-Cas9 technology to become more compatible with the microbiome. Multi-omics integration combines proteomics, metabolomics, and genomes to investigate plant–microbiome interactions. These methods will be useful for sugarcane breeding to increase the overall productivity of sugarcane crops.

8.6 Approach for Microbial Roles in Sugarcane Breeding

Because of their effects on plant development, stress tolerance, and overall production, microbes are essential to improving sugarcane breeding. The use of beneficial microbes, particularly in the rhizosphere (the region of soil near plant roots), has shown potential in complementing sugarcane breeding programs. Figure 8.1 shows the overall approach of using potential microbes in sugarcane breeding to improve sustainable sugarcane crop production.

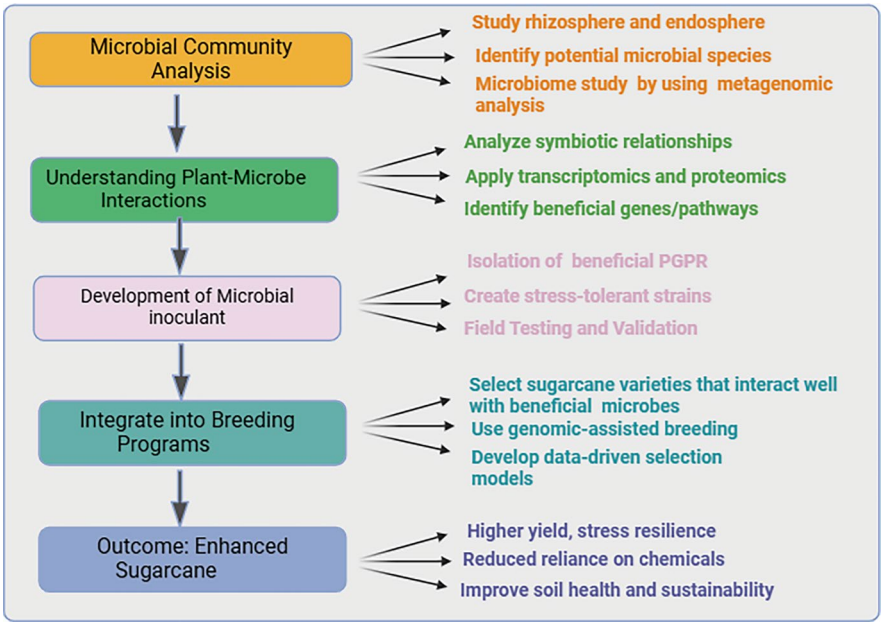

Fig. 8.1 Approach of exploring microbial roles in sugarcane breeding

8.7 Challenges and Future Prospects

The potential role of microbial interactions in sugarcane breeding has gained considerable interest in recent years. With sugarcane being a key crop for sugar production and biofuel, optimizing its yield and resilience is essential. Microbes, especially endophytes and rhizosphere-associated organisms, have shown promise in improving sugarcane's resistance to pests, environmental stresses, and nutrient efficiency. However, leveraging these microbial benefits presents challenges and opens avenues for future research. The microbial communities in the sugarcane rhizosphere and endosphere are highly diverse, making it difficult to identify specific microbes with beneficial traits for breeding. Improved genomic and metagenomic tools can help identify core microbial communities essential for sugarcane growth, stress resistance, and yield improvement. Plant–microbe interactions are highly complex and influenced by environmental factors, soil composition, and the specific sugarcane genotype. Understanding these interactions at a molecular level remains challenging.

Transcriptomics and proteomics can elucidate these interactions, helping breed varieties that form symbiotic associations with beneficial microbes. Although microbial inoculants, like biofertilizers and biopesticides exist, their effectiveness varies based on environmental conditions, soil health, and sugarcane genotype. The lack of stability and consistency is a major challenge. Developing robust microbial inoculants with high ecological adaptability and specificity for sugarcane is essential. This could involve using stress-tolerant microbial strains or microbial consortia that provide synergistic effects. Soil properties can hinder the colonization of beneficial microbes. High salinity, poor drainage, and low organic matter reduce microbial survival rates in the field. Enhancing soil health through organic amendments or soil microbiome engineering could create an environment conducive to microbial colonization and activity, ultimately supporting sugarcane growth.

Integrating microbiome studies into sugarcane breeding makes it possible to identify and select sugarcane genotypes that inherently attract beneficial microbes. Genomic-assisted breeding could be pivotal in creating varieties optimized for microbial interactions. Applying multi-omics (e.g., genomics, transcriptomics, and proteomics) can offer insights into the functional roles of microbes associated with sugarcane. This approach can help identify specific microbial genes or pathways that enhance nutrient uptake, stress tolerance, or disease resistance. Integrating microbes into sugarcane breeding can help reduce reliance on chemical fertilizers and pesticides, promoting sustainable practices. Research can focus on microbial solutions that decrease carbon footprint and enhance soil fertility over time.

8.8 Conclusion

Microbial roles in sugarcane breeding present promising pathways for improving crop resilience, productivity, and sustainability. Exploring microbial roles in sugarcane breeding opens a promising frontier for enhancing crop resilience, productivity, and sustainability. Although there are challenges in microbial diversity, plant–microbe interactions, and the effectiveness of inoculants, advancements in genomic tools, multi-omics, and synthetic biology offer valuable solutions. Developing microbial inoculants that can adapt to diverse environmental conditions, coupled with data-driven can optimize microbial associations and boost sugarcane performance. By integrating microbial approaches into breeding programs, sugarcane cultivation can evolve toward more sustainable and resource-efficient practices, reducing reliance on chemical inputs and fostering long-term soil health. Addressing these challenges with innovative research and sustainable practices could transform sugarcane agriculture, benefiting farmers and the environment.

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Chapter 9
Source–Sink Relationship in Sugarcane and Its Intrinsic Regulatory Mechanisms as a Tool for Plant Breeding

Yeison Mauricio Quevedo-Amaya and Liz Patricia Moreno-Fonseca

Abstract Sugarcane is the primary raw material for sugar, energy, ethanol and other by-products. Although the yield of sugarcane per hectare has been increasing, there has been a plateau in the genetic gains in sucrose yield (kilograms of sucrose per tonne of milled cane). This has led to an increase in sucrose yield per hectare at the cost of higher harvest and processing expenses, impacting the profitability of this agroindustry sector. There is a trade-off between fibre and sucrose production, which limits the increase in sucrose accumulation without affecting fibre and vice versa. Therefore, the main challenge in sugarcane research is to enhance sucrose yield without compromising biomass production. Fibre and sucrose are produced by establishing a source–sink relationship, which involves the interaction of organs responsible for synthesising, transporting and storing carbohydrates. Modern breeding efforts aim to improve processes such as photosynthesis, the transportation, consumption and accumulation of photoassimilates. However, it is not possible to improve these processes without enhancing the complex signalling pathways and feedback regulation that involve both intrinsic and extrinsic factors. Intrinsic factors include regulation by end-products (sucrose) and hormonal signals, such as gibberellins, auxins, cytokinins, ethylene and abscisic acid. Gibberellins and auxins participate in the formation of the sink organ, which is the internode in sugarcane. These phytohormones trigger signalling pathways associated with cell division and elongation and the generation of sink strength through the induction of activity of enzymes, such as invertases and sucrose synthase. However, phytohormones, such as ethylene and abscisic acid, have a synergistic effect and provide major activity during the ripening stage through action on signalling pathways of defoliation, phloem loading and unloading, carbohydrate reallocation and the enhancement of

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activity by sucrose synthesis enzymes, such as sucrose phosphate synthase. This chapter discusses the source–sink relationship in sugarcane and its close relatives, including source strength and sugar movement, sink organ formation and sucrose utilization and accumulation. It critically analyses the entire source–sink relationship, providing an in-depth review of the main bottlenecks associated with phytohormone signalling. Understanding these signalling pathways will be useful in comprehending the dynamics of sucrose accumulation, enabling the identification of critical points that could be targeted in plant breeding to maintain efficient coordination of the entire source–sink relationship to improve sucrose yield without affecting biomass production.

Keywords Photosynthesis · Sugar transport · Phloem loading · Internode · Ripening · Signalling pathways · Hormones · Trade-off

9.1 Introduction

The main challenge of breeding programs is increasing sucrose content, measured in kilograms per ton of milled sugarcane (Chen et al. 2019a, b; Sachdeva et al. 2011). Despite the efforts made in this regard, progress in conventional breeding has been limited. This stagnation is attributed to the polygenic nature of the trait, high non-additive heritability, strong genotype x environment interaction and the trade-off between biomass production and sucrose content (Botha et al. 2013; Chen et al. 2019a, b; Garcia et al. 2018). Moreover, advances in increasing sugar yield per hectare have been based on enlarged cane yield, which reduces profitability due to higher costs associated with harvesting, transportation and milling (Jackson 2005; Sachdeva et al. 2011).

This is particularly evident in energy cane varieties that exhibit a higher carbon allocation toward fibre synthesis at the expense of sugar accumulation. As a result, a higher sucrose yield per hectare is achieved, but at the cost of a greater volume of milled cane (Matsuoka et al. 2014). This phenomenon is explained by the trade-off between fibre and sucrose, where changes in assimilate partitioning favour one over the other, specifically reflecting the balance between carbohydrate source organs and carbohydrate demand organs (source–sink relationship). The source–sink relationship refers to the interaction between organs responsible for carbohydrate biosynthesis and storage organs; effectively coordinating this relationship is essential to enhance crop yield (Chang and Zhu 2017; Foyer and Paul 2001). For over a century, whether the limitation in improving yield lies in the synthesis capacity or carbohydrate utilization has been debated. However, robust evidence suggests that source capacity and sink strength are critical determinants of growth and yield (Rosado-Souza et al. 2023).

Research aimed at improving yield focuses on increasing photosynthesis, to enhance solar radiation capture. Additionally, efforts are targeted at improving sugar transport between source and sink, the size of sink organs and the optimization of sucrose utilization within these organs (Aluko et al. 2021; Botha et al. 2023; Garcia et al. 2018; Hammer et al. 2009; Zheng et al. 2009). Nevertheless, it is important to note that these metabolic fluxes are not regulated by a single enzyme but by complex signalling networks involving multiple enzymes. Therefore, modifying the activity of a single enzyme does not significantly alter the overall metabolic flux or source–sink relationship. Consequently, to boost sucrose content, approaches that enable the manipulation of entire metabolic pathways, enhancing both sink strength and source capacity, must be considered (Botha 2018; Yu et al. 2015).

To achieve the above, it is imperative to elucidate the physiological basis of source–sink regulation at the metabolic level. Identifying genes associated with source–sink regulation may provide insights that facilitate enhanced sucrose content (Mehdi et al. 2024). However, the expression of these genes is regulated by signalling pathways involving phytohormones and sugars (Cunha et al. 2017; Mehdi et al. 2024; Verma et al. 2017), whose expression varies in response to abiotic factors (Lemoine et al. 2013; Verma et al. 2019a, b, 2024a). This understanding is crucial for developing genetic improvement strategies through conventional, transgenic, or gene-editing techniques and optimising agronomic management practices (Afghan et al. 2024; Liang et al. 2024). Therefore, this chapter aims to compile and analyse information on the regulation exerted by intrinsic factors in the source–sink relationship, focusing specifically on sugarcane while drawing on information from other cultivated grasses.

9.2 Source–Sink Relationship

Source organs export photoassimilates to sink organs to support three basic processes: (i) maintenance respiration, (ii) growth respiration and (iii) storage (Botha et al. 2013). This process is regulated by sink strength, which is a concentration gradient between the source, flow and sink that prevents feedback inhibition on photosynthesis by the final product (McCormick et al. 2006; Wang et al. 2013). Sugarcane has a unique source–sink system that stores extremely high concentrations of sucrose (exceeding 650 mM). This storage occurs in the parenchymal tissue of the stem rather than in terminal organs (McCormick et al. 2009; Welbaum et al. 1992). Moreover, it is known that the sugarcane root system also acts as a sink organ, importing sucrose for cell wall formation, maintenance and storage (Inman-Bamber et al. 2011), in addition to translocate to late-formed stems or ‘suckers’ (Hartt et al. 1963). For the purposes of this chapter, we will focus on the internodes of mature stems as sink organs. In these cases, sucrose accumulation happens simultaneously with their formation, that is, during internode elongation (Bonnett 2013).

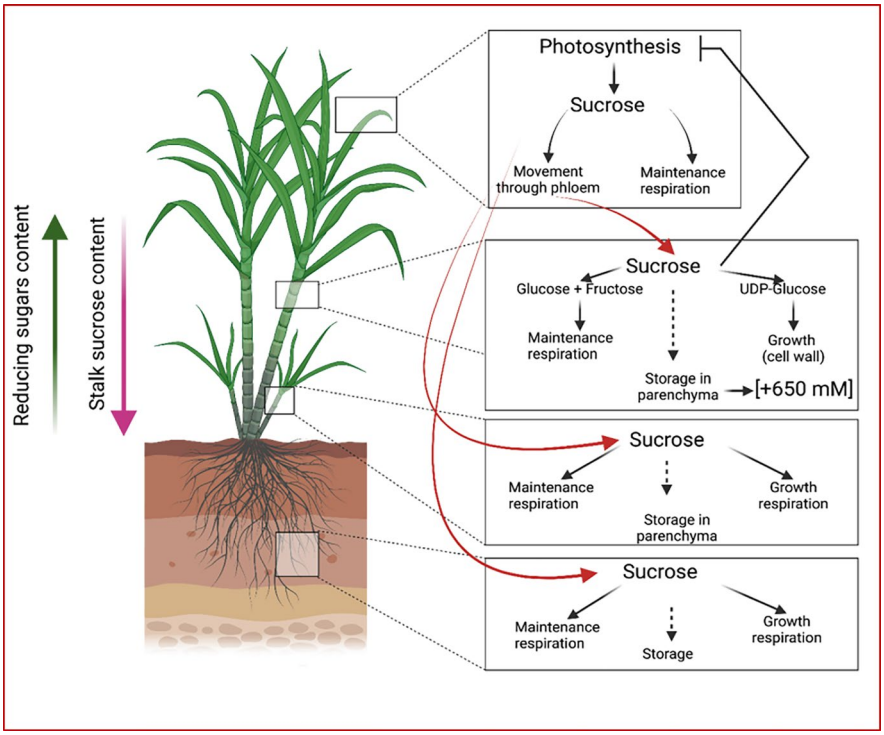

Fig. 9.1 The diagram illustrates the movement and usage of sucrose from the source to the sink organs in a sugarcane plant. Red lines represent long-distance sucrose movement, while black lines indicate metabolism within each organ. Dotted black lines show the final destination of sucrose in each organ. Truncated lines indicate downregulation. Additionally, the green arrow on the left signifies the gradient of reducing sugar content within the stem, from lower to higher content. In contrast, the purple arrow indicates the gradient of sucrose content within the stem, from lower to higher content. (Created with BioRender.com)

This means that the process is continuous, beginning with the appearance of the first internode and extending until harvest (Muchow et al. 1996; Quevedo et al. 2023). There is spatial variability along the stem in terms of sucrose content, with higher concentrations in the basal internodes and lower concentrations in younger or apical internodes due to the sink organ's filling time (McCormick et al. 2006). Figure 9.1 describes the relationship between source and sink in sugarcane.

9.3 Source Capacity

The sugarcane plant exhibits a C_4 photosynthetic metabolism with a classical anatomy and biochemistry characterized by the use of NADP-Malic enzyme to decarboxylate malate in the chloroplasts of bundle sheath (BS) cells (Sage et al. 2013).

Consequently, sugarcane is considered one of the most efficient plants in converting solar energy into chemical energy (Wang et al. 2013; Verma et al. 2020a; Chen et al. 2020a, b). Although photosynthesis in C_4 plants is considerably more efficient than in C_3 plants, there is still substantial room for improvement, as the efficiencies achieved today are far below theoretical limits (Sales et al. 2021). According to simulation models, the critical points in C_4 photosynthesis under non-stressful conditions are electron transport in the photochemical phase, malate decarboxylation via NADP-ME and carbon fixation by Rubisco (Wang et al. 2021). Altering one of these reactions could influence the entire process (Pignon and Long 2020). Therefore, the following sections will address these topics and explore approaches to improve photosynthesis.

9.3.1 Energy Capture and Electron Transport

The first step of phase 1 in photosynthesis is the capture of light photons by antenna complexes. Cytokinins (CK) are molecularly related to chloroplast organogenesis, chlorophyll synthesis and stomatal conductance (Ahanger et al. 2020). Additionally, under stress conditions, CK delays leaf senescence by increasing the transcription of invertase genes at the mitochondrial level, leading to sucrose degradation, amplifying glycolytic activity and consequently reducing feedback inhibition on photosynthesis (Gujjar et al. 2021). In this way, CKs enhance carbohydrate synthesis, increasing growth respiration and promoting leaf expansion, thereby boosting source organ strength (Glanz-Idan et al. 2020).

The enzyme isopentenyl transferase (IPT) catalyses a rate-limiting step in CK biosynthesis (Miyawaki et al. 2006). In corn and rice, overexpression of IPT increases endogenous CK levels, resulting in higher chlorophyll concentrations, enhanced photosynthesis, delayed senescence and reduced catabolism, thereby improving yield (Peleg et al. 2011; Song et al. 2022). In sugarcane, CK oxidase silencing or IPT overexpression leads to greater tolerance to water deficit or cold through mechanisms that protect the photosynthetic apparatus and enhance sink strength by upregulating invertase activity (Belintani et al. 2012; Gupta et al. 2023; Masoabi et al. 2023; Zarea and Karimi 2023).

Another phytohormone involved in photosynthesis regulation is brassinosteroids, triggered by the activation of the transcription factor BZR1 via the target of rapamycin (TOR) kinase, a signalling cascade linked to chloroplast formation and chlorophyll biosynthesis (Song et al. 2021; Zhang et al. 2016a, b). TOR activity is promoted by glucose or sucrose at a concentration of 15 mM, which induces sugar utilization in glycolytic processes. At the same time, it is inhibited under stress conditions or low carbon availability due to SnRK1 (SNF1-related protein kinase 1) (Sharma et al. 2020). TOR upregulation enhances solar radiation capture, improving photosynthesis and growth (Zhang et al. 2016a, b, 2022). This energy is then transferred to the electron transport chain, regulated mainly by the cytochrome b6f complex (Foyer and Paul 2001). This complex is a promising target for improving

photosynthesis through genetic engineering (Malone et al. 2021; Verma et al. 2024a, b, c). Overexpression of the Rieske FeS protein was found to raise the amount of cytochrome b6f complex in both BS cells and mesophyll, which improved the electron transport rate and, consequently, photosynthesis efficiency (Ermakova et al. 2019). These results in a model C_4 plant support the hypothesis that improving electron transport or alleviating its limitations is a viable strategy for enhancing photosynthesis in C_4 plants.

9.3.2 C_4 and C_3 Cycle

A limiting step in the C_4 cycle is the malate decarboxylation, which releases CO_2 and produces pyruvate. This process is regulated by the enzyme NADP-ME (Sage et al. 2013; Wang et al. 2021). In corn, the expression of ZmC4-NADP-ME is controlled by transcription factors (TF), particularly ZmNF-YC2 and ZmbHLH157, which may be related to brassinosteroid signalling (Gao et al. 2023). This could explain the boost in photosynthesis and growth in C_4 plants driven by brassinosteroids and how manipulation of this pathway can lead to enhanced source strength (Kočová et al. 2010; Song et al. 2021). Some studies have found that the PEP/Rubisco ratio is suboptimal due to an excessive allocation of resources toward PEP (Pignon and Long 2020). Therefore, it has been hypothesized that an 8% reduction in PEP and a 15% increase in Rubisco could lead to a 12% improvement in photosynthesis and water use efficiency in species such as sugarcane without increasing nitrogen consumption (Sales et al. 2021).

However, the carboxylation of ribulose-1,5-bisphosphate by Rubisco represents one of the critical steps in the C_3 cycle (Spreitzer and Salvucci 2002). In recent years, genetic improvement in C_4 plants has focused on increasing Rubisco content and its catalytic activity (Sales et al. 2021). Overexpression of the Rubisco assembly factor chaperone (RAF1) and its two subunits was found to increase CO_2 capture and growth by 30% despite low Rubisco activation by Rubisco activase (Rca) (Salesse-Smith et al. 2020). Rca removes sugar-phosphate residues from Rubisco's active sites, a function limited by its high thermolability (Perdomo et al. 2017; Spreitzer and Salvucci 2002). In sugarcane, photosynthesis is reduced under high-temperature conditions due to photochemical and biochemical effects (Liu et al. 2020; Jagadesh et al. 2024; Huang et al. 2024). With regard to improving source strength, it has been found that the Rca-α isoform of Rca elevates its expression in response to high-temperature stress in sugarcane, maintaining Rubisco activation and, consequently, the CO_2 fixation rate (Kim et al. 2021). To sustain source activity, increasing Rca thermotolerance (Wijewardene et al. 2021) or maintaining an optimal ratio between Rubisco and Rca is necessary.

The capacity for canopy photosynthesis, or source capacity, decreases significantly as the crop's life cycle progresses (Verma et al. 2020b, c, 2022a, b). Higher values are observed in 4-month-old plants than in 8-month-old plants (Allison et al. 1997; Cock et al. 1997). This decline is mainly due to the reduction in leaf nitrogen

content, which is attributed to early nitrogen fertilization and is compounded by the inefficient use of nitrogen in photosynthesis, a common trait in sugarcane (Allison et al. 1997; Botha 2018; Anas et al. 2021a, b).

On the contrary, source strength is upregulated in low sugar concentrations. This occurs when sink organs increase their respiratory and storage capacity in response to excess carbon. In an opposite scenario, a high concentration of sucrose or hexoses at the foliar level due to insufficient sink strength or a low carbohydrate export rate from the source triggers the differential expression of 74 genes. In this case, downregulation of genes related to the photo phase and C_3 and C_4 cycles is observed, while genes involved in assimilate partitioning, cell wall synthesis and stress are upregulated (McCormick et al. 2008; Rossi et al. 2015). This indicates fine coordination between both organs through sugar-associated signalling pathways (Rolland et al. 2006). Signalling pathways associated with feedback between the sink and source, both dependent and independent of hexokinases, have been described, including those linked to trehalose-6-phosphate (T6P) and sucrose-induced regulation of translation (SIRT) (McCormick et al. 2008; Mehdi et al. 2024; Park et al. 2005; Watt et al. 2005). These mechanisms will be described below.

9.3.3 Trehalose Metabolism

T6P is a sugar-level sensor that triggers the downregulation of genes related to photosynthesis (McCormick et al. 2006; Yadav et al. 2014). T6P is produced in conditions of excess glucose, where HXK1 phosphorylates glucose to form glucose-6-phosphate, which is then converted into T6P by trehalose phosphate synthase (TPS) (Mehdi et al. 2024; Moore et al. 2003; Yanagisawa et al. 2003). T6P interacts with SnRK1, forming the T6P-SnRK1 complex, which induces leaf senescence (Asim et al. 2022). Activation of this pathway results in the differential expression of 74 genes, with genes involved in the photo phase and C_3 and C_4 cycles being downregulated (McCormick et al. 2008).

In corn, overexpression of trehalose-6-phosphate phosphatase (TPP) reduces T6P levels, resulting in increased sucrose content in the ears, leading to higher yields (Nuccio et al. 2015; Ponnu et al. 2011). Meanwhile, in sugarcane, markers associated with TPS6, 7 and 11 have negatively affected early and normal sucrose accumulation (Saavedra-Díaz et al. 2024). Therefore, manipulating trehalose metabolism through the overexpression of TPP boosts sucrose content and reduces T6P levels without affecting plant growth (Gabriel et al. 2021). The transcription of TSP1, TPP7 and SnRK1A in sink organs is upregulated by basal abscisic acid (ABA) levels, enhancing the sink strength of the internode (Chen et al. 2019a, b). Manipulating the metabolic pathways surrounding trehalose synthesis is an intriguing trait, given the potential for genetic enhancement to increase yields in C_4 plants (Gabriel et al. 2021; Nuccio et al. 2015).

9.3.4 Sucrose-Induced Regulation of Translation (SIRT)

SIRT is a recently proposed hypothetical regulatory mechanism of the source–sink relationship in sugarcane (Mehdi et al. 2024). SIRT is triggered by a specific sucrose concentration, inhibiting the translation of bZIP TFs, such as Scbzip44. This results in the downregulation of asparagine synthetase (ASN) and a subsequent decrease in the activation of the sucrose synthesis pathway. Specifically, the activity of sucrose phosphate synthase (SPS) and sucrose phosphate phosphatase (SPP) is reduced (Thalor et al. 2012). The repression of Scbzip44 translation is due to the presence of an SC-uORS (sink organ response system) in the 5′ leader region of the open reading frame (ORF), which acts as a sucrose sensor (Rolland et al. 2006; Thalor et al. 2012). In *Nicotiana tabacum*, the ORF of the transcription factor Atbzip11 was modified and subsequently overexpressed, resulting in a threefold increase in sucrose compared to the control due to reduced feedback between source and sink (Thalor et al. 2012). Therefore, modifying the ORF of these TFs in sugarcane was hypothesized to eliminate sucrose-mediated feedback, increasing stem sucrose content (Mehdi et al. 2024).

This same goal could be achieved by temporarily redirecting sucrose use and allocation toward starch generation, as enhanced T6P content promotes starch synthesis, increasing the transcription of bzip11 and ASN (Griffiths et al. 2016). Another option is the synthesis of sugars, such as isomaltulose, that do not downregulate photosynthesis. In sugarcane transformed to overexpress sucrose isomerase, sugar accumulation can be doubled compared to a conventional plant (Wu and Birch 2007). Reducing the source strength during the final ripening stage can enhance sugar yield. It stimulates defoliation, resulting in fewer leaves entering the harvester. This reduces extraneous plant material and improves sugar recovery indicators in the factory. Varieties with high natural defoliation are characterized by cell wall modifications produced by enzymes such as cellulases and pectinases during the early and intermediate stages of maturation, leading to programmed cell death (Hu et al. 2023a, b). This process is driven by elevated ethylene (ET) and abscisic acid (ABA) synthesis, as well as reduced auxin (AUX) accumulation in the abscission zone (Gao et al. 2015, 2017; Hu et al. 2023a, b; Xu et al. 2021). Transcriptomic studies have identified 138 genes related to ABA response, 34 genes responsive to ET, 17 genes associated with leaf senescence and 17 genes involved in cell wall modification (Xu et al. 2018).

Coordination between source and sink is essential to boost sucrose accumulation in sugarcane internodes to prevent feedback on photosynthesis caused by sucrose accumulation in sink organs and, consequently, in the source. This coordination occurs through three signalling pathways involving T6P, TOR and SIRT. It is proposed that temporarily redirecting sucrose use and allocation toward starch generation, manipulating trehalose metabolism, synthesizing sugars such as isomaltulose, or modifying the ORF of bZIP transcription factors with SC-uORS could produce plants with high sucrose content by eliminating sucrose-mediated feedback. Additionally, promoting natural defoliation would indirectly affect sugar yield (Fig. 9.2). However, these strategies must be accompanied by carbohydrate flow improvements and sink organ utilization.

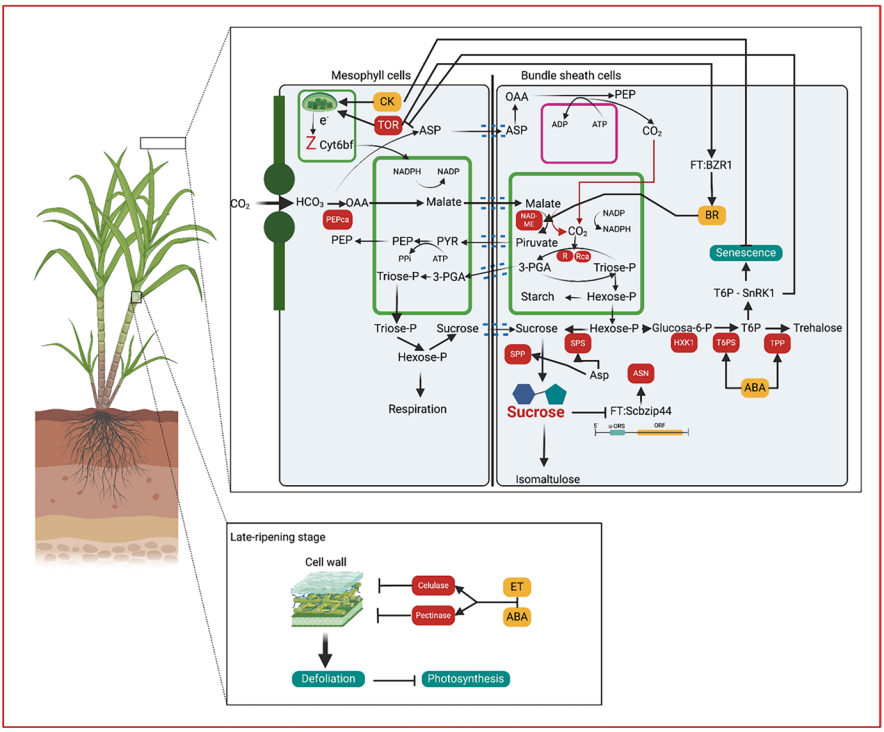

Fig. 9.2 Signalling model of source–sink regulation in grasses such as sugarcane, rice and corn. The cytoplasm, chloroplasts and mitochondria are delineated by black, green and purple lines, respectively. Blue dashed lines indicate intercellular communication via plasmodesmata. Black arrows show upregulation and truncated lines indicate downregulation. Red rectangles refer to enzymes involved in each process. Green rectangles indicate macro processes related to the preceding compound. Yellow rectangles highlight the hormones involved. *HXK1* Hexokinase 1, *T6P* Trehalose-6-phosphate, *T6PS* Trehalose-6-phosphate synthase, *SPS* Sucrose phosphate synthase, *SPP* Sucrose phosphate phosphatase, *ASN* Asparagine synthetase, *Asp* Asparagine, *TF* Transcription factor, *ORF* Open reading frame, *SnRK1* Sucrose non-fermenting-1 related kinases, *TOR* Target of Rapamycin, *PEPca* Phosphoenolpyruvate carboxylase, *R* Ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco), *Rca* Rubisco activase, *CK* Cytokinin, *BR* Brassinosteroid, *ABA* Abscisic acid, *ET* Ethylene. (Created with BioRender.com)

9.4 Sucrose Transport

Photoassimilates that are not used in the growth and leaf maintenance respiration are exported to other organs through phloem sieve elements (SE) (Braun et al. 2014; Osorio et al. 2014). This occurs due to a hydrostatic pressure gradient caused by the flow of water and dissolved photoassimilates, maintained by the loading and unloading processes in SEs and companion cells (CC) (Grof et al. 2013; Holbrook and Zwieniecki 2005; Rae et al. 2005). Sucrose transport from source to sink involves several processes: (i) short-distance transport from mesophyll cells to CC; (ii) long-distance transport, which involves sucrose loading into the SE-CC complex and its

movement through the phloem; (iii) sucrose unloading into the sink organ. These processes are regulated by the activity of transporters and mineral elements such as phosphorus, magnesium and potassium (Aluko et al. 2021). Furthermore, biotic factors can harm or obstruct the phloem, for example, through the accumulation of callose (Lemoine et al. 2013).

In sugarcane, sucrose is loaded into the phloem in small and intermediate veins, then transported long-distance through one of the four lateral veins and finally reaches the sheath leaf, from where it is transported to the apical meristem of the stem at an average speed of 84 cm per hour (Braun 2022; Hartt et al. 1963; Slewinski et al. 2013). This section compiles relevant information on the mechanisms of phloem loading and unloading in sugarcane and similar plants and key aspects to consider in designing new strategies to improve yield.

9.4.1 Phloem Loading

In short-distance transport at the leaf level, sucrose moves cell-to-cell via the symplast or apoplast until it reaches the BS cells, which are located near the veins (Aluko et al. 2021; Braun et al. 2014). For sugarcane, evidence suggests that phloem loading can be symplastic or apoplastic due to variability in the level of suberization around the vascular bundles (Chen et al. 2022). However, in this context, we will concentrate on apoplastic transport, as it is considered the primary mechanism in sugarcane (Grof et al. 2013; Rae et al. 2005; Robinson-Beers and Evert 1991). In apoplastic transport, sucrose moves from the mesophyll to the BS cells or parenchyma (PP) cells through the symplast (Grof et al. 2013; Robinson-Beers and Evert 1991). Since there are no plasmodesmata between PP and CC, transporters, such as SUT/SUC and SWEET, are required (Braun et al. 2014; Dhungana and Braun 2022; Wang et al. 2022).

Pre-loading of the phloem involves generating a concentration gradient between the cytoplasm of mesophyll and PP cells to facilitate symplastic transport. This secondary active transport process involves TST transporters, which act as antiporters with H+ and a proton pump that generates the driving force for sucrose movement (Braun 2022). Activation of TST2 occurs through a highly conserved signalling cascade in angiosperms involving CALCINEURINEB-LIKE2, which recruits CLB-INTERACTING PROTEIN KINASE6 and subsequently phosphorylates TST2, triggering the entry of sugars into the vacuole (Dhungana and Braun 2022; Deng et al. 2020). In sugarcane, the transporters SsTST2a and SoTST2a are highly expressed in leaves, suggesting their involvement in the pre-loading process (Dhungana and Braun 2022).

Once sucrose has reached the PP cells via the symplast, it must exit into the apoplast and enter the CCs, a process mediated by SUT/SUC and SWEET transporters, respectively (Braun 2022). In *Saccharum spontaneum*, 22 genes from the SWEET family have been identified, of which SsSWEET13c is most closely related to the bidirectional transport of glucose, fructose and sucrose and is considered a

duplicated gene alongside SWEET13a and b (Hu et al. 2018). SvSWEET13a and b are mainly expressed in BS and PP, particularly in small veins, and show a higher affinity for sucrose (Chen et al. 2022). Meanwhile, the expression of SsSWEET13c in *S. spontaneum* is more pronounced in the apical region of leaves, where the highest photosynthesis rate is achieved, and its overexpression resulted in increased export of photoassimilates from the leaves. This transporter is upregulated and downregulated by 10 and 4 TF, respectively. The most relevant regulators are SsbHLH34 (positive) and SsbZIP44 (negative), responding to the circadian cycle and sucrose levels (Hua et al. 2022). These SWEET regulators offer an excellent opportunity to manipulate phloem transport and reduce feedback on photosynthesis, potentially resulting in increased crop yield (Chen et al. 2022).

Sucrose must enter in the CC-SE complex after transporting from PP to the apoplast. In sugarcane, this process occurs against the concentration gradient, requiring the action of an H^+ ATP_{ase} to activate SUT/SUC transporters, which function as a sucrose/H^+ symporter, importing sucrose from the apoplast into the CC-SE complex (Baker et al. 2016; Braun 2022; Carpaneto et al. 2005; Eom et al. 2015; Xu and Liesche 2021). This active transport type requires ATP to activate H^+ ATP_{ases} (Dreyer et al. 2017). When ATP availability is limited, K^+ can partially replace the ATP_{ase} function by accumulating in the apoplast, generating a more positive charge that facilitates SUT/SUC action. This process occurs through the transport of K^+ from the CC-SE cytosol to the apoplast, mediated by AKT2/3 channels (Deeken et al. 2002; Dreyer et al. 2017). On the other hand, Mg^{+2} deficiencies reduce the availability of Mg-ATP, decreasing ATP_{ase} activity and, consequently, SUT/SUC activity (Lemoine et al. 2013; Marschner et al. 1996). In this context, mineral elements such as phosphorus, magnesium and potassium are essential for sucrose mobility from source to sink.

It has been proposed that a bypass in the Krebs cycle elevates ATP production, which is essential for activating SUT/SUC. This bypass occurs through the γ-aminobutyric acid (GABA) pathway, synthesized from glutamate for succinate production (McIntyre et al. 2021). Succinate is converted into fumarate in a reaction catalysed by succinate dehydrogenase, reducing FAD to $FADH_2$, which enters the electron transport chain and generates the proton gradient needed for ATP synthesis (Gnaiger 2023). Although there is no direct metabolic connection between GABA and CK, it has been observed that plants with high concentrations of trans-zeatin show more significant accumulation of GABA (Merewitz et al. 2012). Similarly, higher concentrations of Krebs cycle metabolites have been found in high-sucrose sugarcane varieties (Perlo et al. 2020). This could support the hypothesis that improving phloem loading is linked to increased ATP synthesis for SUT/SUC activation.

SUT/SUC genes in corn and sugarcane share 95% identity (Rae et al. 2005). ZmSUT1 is expressed in BS, CC and PP, mainly importing sucrose from the apoplast into CCs (Baker et al. 2016; Slewinski et al. 2013). In sugarcane, six SUT/SUC genes have been identified (Dhungana and Braun 2022). SUT1, SUT2 and SUT5 appear to be the SUT family genes most involved in phloem loading due to their high expression in leaves (Dhungana and Braun 2022; Zhang et al. 2016a, b).

The expression of ShSUT1 and ShSUT5 is upregulated by the TF ShbZIP9 isoform X2, which is highly expressed in high-sucrose varieties and downregulated by miR169 in low-sucrose varieties (Wang et al. 2022). On the other hand, the presence of SUT4 is positively correlated with sucrose accumulation under high soil moisture conditions (Saavedra-Díaz et al. 2024).

9.4.2 Sucrose Flow Through the Phloem

Sucrose flow through the SEs is governed by a pressure gradient that exists between the source and the sink organ, known as Münch's pressure flow hypothesis (Grof et al. 2013; Münch 1930). This occurs because, as sucrose enters the collection region of the phloem, there is a decrease in water potential, causing water to enter from the xylem by osmosis, generating movement along the water potential gradient. When sucrose is unloaded in the sink organ, the water potential of the phloem increases, resulting in water diffusion outward and, consequently, a loss of pressure (Braun et al. 2014; Patrick 2013). Previous research has revealed a linear relationship between phloem bundle size and sucrose accumulation capacity in sorghum. For every additional 271.27 μm^2 of phloem area, one-degree brix rises (Kanbar et al. 2021). This finding underscores the importance of phloem bundle size in sucrose accumulation and highlights its relevance in the source–sink relationship in sucrose-accumulating grasses.

9.4.3 Phloem Unloading

Phloem unloading transfers solutes from the SE-CC complex to the stem parenchyma cells (SPC), where they are stored or used for growth (Braun et al. 2014; Weber et al. 1997). In sugarcane, the sink organs are vegetative in nature so that phloem unloading can be either symplastic or apoplastic (Braun 2022; Grof et al. 2013; Rae et al. 2005; Wang et al. 2013). As internode ripening progresses, the vascular bundles in the stem become surrounded by a layer of cells with suberized and lignified cell walls, forming a barrier to apoplastic movement and preventing sucrose leakage (Jacobsen et al. 1992; Rae et al. 2005). As a result, in mature internodes, symplastic unloading becomes more relevant (Patrick 2013). To maintain the concentration gradient, sucrose must exit the CCs into the apoplast, a process mediated by SWEET transporters (Braun 2022).

Both apoplastic transport in immature internodes and symplastic transport in mature internodes are considered active transport due to the involvement of transporters, in a process similar to phloem loading. This process involves SUC/SUT and SWEET transporters along with cell wall acid invertase (CWI) (Wang et al. 2013). The first step to move sucrose from CC to SPC via the apoplast is mediated by SWEET, which can also facilitate hexose and sucrose movement (Chen et al. 2023).

In sugarcane, SoSWEET1a, 4a, 4b, 13a, 13b, 13c and 16a have been identified as sucrose transporters from CCs and sclerenchyma cells to the apoplast (Hu et al. 2018). In sweet sorghum, SbSWEET3 and 4 transport sucrose to the apoplast, which is then imported into PP or SPC by the symporter SbSUT1, 2 and 4 (Bihmidine et al. 2013; Mizuno et al. 2016). It is essential to mention that SbSUT1 and SbSUT5 are in the plasma membrane, while SbSUT4 is found in the tonoplast membrane. Additionally, SbSUT1's affinity for sucrose depends on pH and membrane potential, unlike SbSUT5 (Milne et al. 2017). Nevertheless, the expression of SbSUT1, SbSUT2 and SbSUT6 does not vary between genotypes with different sucrose accumulation capacities (Kanbar et al. 2021). In sugarcane internodes, high expression of SUT1, 2 and 4 has been identified, suggesting their involvement in sucrose transport from the apoplastic space to SPCs (Reinders et al. 2006; Zhang et al. 2016a, b), specifically SUT4 in SPCs and SUT1 in BS cells (Casu et al. 2015).

In sugarcane, the expression of SUC/SUT in both source and sink organs is regulated by ET signalling (Chen et al. 2019a, b; Cunha et al. 2017; Neris et al. 2022). Meanwhile, in rice and corn, ABA basal concentrations have been observed to augment the expression of SUT1 and SWEET15a in sink organs (Chen et al. 2019a, b; Liu et al. 2022). This suggests an important role for these two phytohormones in sugar transport and a possible interaction that will be described later.

In apoplastic transport, CWI plays a crucial role by creating a concentration gradient through energy expenditure. This process triggers phloem unloading in the internodes (Wang et al. 2013). CWI is primarily found in immature internodes, where sucrose hydrolysis reduces the sucrose ratio, improving the energy demand of the internodes and reducing the low-demand signal caused by sucrose feedback (Chandra et al. 2012). After hydrolysis, hexoses can enter the SPC via SUT/SUC (Milne et al. 2017). While CWI is regulated by different phytohormones, such as gibberellins (GA), auxins (AUX), ABA and ET, its role is more critical in sink organ formation or sucrose utilization, which is why its regulation will be discussed in more detail in subsequent sections. Figure 9.3 shows the sucrose loading and unloading processes in the phloem and the movement towards the SPC.

9.5 Internode Development

In sugarcane, the stems are the sink organs. They are formed during stages 3 and 4 of the BBCH scale, and their development is continuous from 3 months of age until harvest in Colombian conditions (Bonnett 2013; Muchow et al. 1996; Quevedo et al. 2023). The six youngest internodes are in constant cell division and elongation. An elongating internode is divided into four parts, each with differentiated physiological behaviour (Wei et al. 2019; Yanagui et al. 2022; Li et al. 2024; Malviya et al. 2024). The following is a description of each zone according to Yanagui et al. (2022):

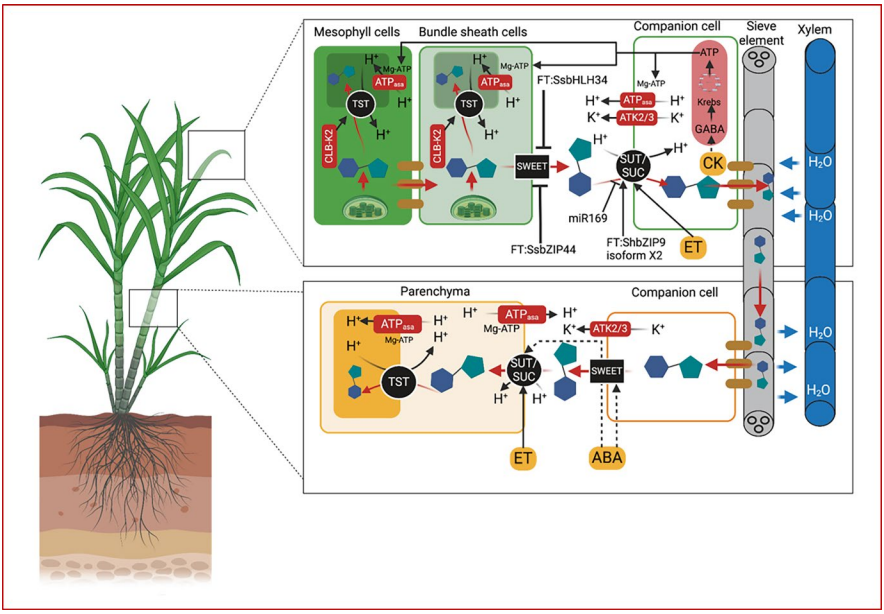

Fig. 9.3 The process of loading and unloading sucrose in the phloem of sugarcane plants involves several key components. Sucrose molecules flow from the source cells (mesophyll and bundle sheath) to the sink (stem parenchyma), as indicated by red arrows. The movement of H^+ and K^+ through transporters or proton pumps is represented by black arrows. Water movement from the xylem to the phloem and vice versa is depicted by blue arrows. Sucrose molecules are represented by hexagons and pentagons, while intercellular connections via plasmodesmata are shown by brown rectangles. The pink oval represents the mitochondrion, while black rectangles and circles refer to sugar transporters such as SWEET, SUT/SUC and TST. Key proteins in each process are highlighted by red rectangles, and yellow rectangles indicate the hormones involved. Solid lines represent upregulation; truncated lines show downregulation, and dashed lines indicate a hypothetical relationship. The hormones involved in the process include CK (cytokinin), ET (ethylene), ABA (abscisic acid) and GABA (γ-aminobutyric acid), as well as TF (transcription factor). (Created with BioRender.com)

- *Cell division zone:* This area shows high expression of genes related to RNA, DNA and protein synthesis, as well as energy production and cellular processes.
- *Elongation zone:* In the elongation zone, there is increased transcription of genes related to expansins, sugar, sulphate, lipids and proline transporters.
- *Transition zone:* This zone shows high expression of the phenylalanine ammonia-lyase (PAL) gene related to phenylpropanoids; GT43, associated with xylan synthesis; and UDP-xylose synthase (UXS), responsible for the first step in the synthesis of pectin, hemicellulose and glucuronoarabinoxylan polymers. In other words, metabolic activity is focused on secondary cell wall formation.

- *Ripening zone:* A high lignin content is found in this part of the internode. Therefore, like the transition zone, high PAL, GT43 and UXS expression are observed. Additionally, the expression of cellulose synthase (CesA), polyubiquitin, metallothionein and S-adenosylmethionine synthetase genes is detected, which are related to cellulose biosynthesis, protein degradation and ET synthesis, respectively. Secondary cell wall formation and lignification in this zone are directly related to phloem unloading and sucrose accumulation.

Finally, differences in the expression of MYB TFs were observed along the internode. MYB4, MYB63, MYB32 and MYB85 show high expression in the ripening and transition zones and are considered regulators of the phenylpropanoid pathway. However, MYB103, related to CesA, presents high transcription in the elongation and transition zones. These TFs have the potential to act as master regulators in internode formation and may be used for the selection of varieties through molecular markers or genetic engineering.

9.5.1 Internode Growth

Internode growth is regulated by a complex signalling network involving several phytohormones, including GA, AUX and ET (Chen et al. 2020a, b; Garcia et al. 2018; Nagai et al. 2020; Wei et al. 2019). These hormones are considered master regulators of sink strength, which is the capacity to import photoassimilates and is determined by the size, metabolic activity and growth rate of the organ (Bihmidine et al. 2013; Guo et al. 2021, 2022). Below is a description of the role of each phytohormone in internode growth.

9.5.1.1 Auxins

AUX are related to the source–sink relationship due to their role in sink organ growth (Yu et al. 2015). They are involved in cell division, elongation and differentiation, with a particularly important role in acid growth (Botha et al. 2013). AUX and GA mediate cell elongation; nonetheless, GA signalling might occur after the acid growth reaction is induced by AUX, as acid growth activation is faster than GA action (Yang et al. 1996). Thus, in the cell division region of the internode, there is increased AUX activity, likely regulating the expression of genes related to the cell cycle, organization, development and differentiation, as well as protein biogenesis and modification and nucleic acid synthesis (Yanagui et al. 2022). However, in the elongation zone, there is elevated expression of α- and β-expansins, which are involved in acid growth (Botha et al. 2013; Sampedro and Cosgrove 2005; Yanagui et al. 2022).

9.5.1.2 Gibberellins

GAs influence the balance between source and sink by promoting the formation of sink organs and photosynthesis. This leads to increased phloem loading and translocation of assimilates (Iqbal et al. 2011). In sugarcane, internode elongation is related to GA-mediated signalling cascades, as they influence the expression of genes involved in cellulose, hemicellulose, lignin synthesis, respiration and oxidative phosphorylation (Chen et al. 2020a, b; Roopendra et al. 2018). Nonetheless, the duration of internode elongation is controlled by thermal time or accumulated degree days (Botha et al. 2023).

GA-induced signalling is downregulated by the expression of DELLA proteins, one of which is encoded in sugarcane by the ScGAI gene. Suppression of this gene resulted in a significant rise in stem growth, phytomer emission and photosynthesis rate compared to plants overexpressing ScGAI (Garcia et al. 2018). Nevertheless, exogenous application of GA_3 during the rapid growth stage leads to enhanced transcription of genes associated with the biosynthesis of cutin, suberin, waxes and photosynthesis, resulting in a higher growth rate, internode length and plant height (Chen et al. 2020a, b; Singh et al. 2020; Sharma et al. 2021, 2022). Additionally, there is a rise in the relative expression of carbohydrate-metabolizing enzymes, such as sucrose synthase (SuSy), soluble acid invertase (SAI) and CWI between 50 and 80 days after application, indicating sugar consumption to increase internode volume and, consequently, sink strength. Subsequently, there is a significant boost in SPS expression, leading to a substantial enhancement in sucrose content in the middle and apical internodes of the stem (Nguyen et al. 2019; Rai et al. 2017; Verma et al. 2017). At the commercial level, it has been reported that exogenous application of GA_3 enlarges stem volume and thus sink strength, resulting in yield gains of up to 16 tons of cane per hectare without negatively affecting juice quality, augmenting sugar yield by up to 2 t ha^{-1} (Botha et al. 2023; Huang et al. 2022; Singh et al. 2019; Moore 1980; Moore and Buren 1978).

As described earlier, GA signalling during the rapid growth stage positively impacts sink strength and sucrose accumulation. GA signalling needs to be downregulated during the ripening stage as the energy demand for growth respiration decreases, as evidenced by reduced invertase activity (Leite et al. 2011). This drop in the demand for photoassimilates for fibre production in new internodes and leaves in the apices does not significantly reduce source strength, which enhances sucrose accumulation in percentage terms (Inman-Bamber et al. 2008). To achieve this downregulation, the conversion of GA_{20} to GA_1, one of the bioactive forms of GAs, is inhibited by the exogenous application of a growth regulator such as trinexapac-ethyl (Ayele et al. 2023; Resende et al. 2001; van Heerden 2014; van Heerden et al. 2015). Furthermore, under favourable climatic conditions for ripening, such as low air temperature and moderate water deficit (Verma et al. 2020d,e, 2021a,b), GA downregulation occurs through DELLA proteins, meaning the exogenous application of these regulators does not lead to higher sucrose accumulation (Spaunhorst et al. 2019). In contrast, when climatic conditions are unfavourable, applying a growth regulator is necessary to boost sucrose content (Sandoval-Pineda and

Villegas-Trujillo 2023); alternatively, planting varieties with downregulated growth during the ripening stage is recommended (Song et al. 2019; Oliveira et al. 2022; Quevedo et al. 2023).

9.5.1.3 Ethylene

In sugarcane, the main function of ET lies in inducing defoliation, reducing growth, promoting stem ripening and controlling flowering (Alessio et al. 2018; Ayele et al. 2021; Cunha et al. 2017; Gao et al. 2015; Terence 2003). Therefore, it is considered a key regulator in the source–sink relationship of this crop. ET acts by downregulating growth, inducing the expression of genes related to IAA conjugation, such as *GH3–1 and GH3–2*, inhibiting TF, such as phytochrome-interacting factor and deactivating GA_1 and GA_4 due to elevated transcription of gibberellin 2-oxidase ($GA2_{ox}$) and a DELLA protein. This reduces apical dominance and delayed flowering, which enhances sucrose content (Chen et al. 2019a, b; Cunha et al. 2017; Neris et al. 2022). For this reason, ethephon, a substance that releases ET by activating the ACC oxidase enzyme, is widely used as a growth regulator during the ripening stage to stimulate sucrose accumulation in immature internodes by up to 60% more than untreated plants (Alessio et al. 2018; Almeida-Silva and Maitto 2012; Chen et al. 2019a, b; Cunha et al. 2017; Neris et al. 2022). Figure 9.4 illustrates the process of sink organ formation, and the key regulatory mechanisms described.

9.6 Sucrose Use and Storage in the Internode

The enhancement of sucrose content (kilograms of sucrose per tonne of cane) in sugarcane remains a primary challenge in physiology and plant breeding research (Chen et al. 2019a, b; Jackson 2005; Sachdeva et al. 2011). Nevertheless, progress has been limited due to the trade-off between biomass production in terms of fibre and the accumulation of sucrose and reducing sugars (Sabatier et al. 2015). This trade-off is closely related to the distribution of photoassimilates toward producing fibre and soluble and reducing sugars (Matsuoka et al. 2014). It is estimated that two-thirds to three-quarters of the fixed carbon synthesises structural carbohydrates such as cellulose and hemicellulose (Garcia et al. 2018). This is linked to the use and storage of sink organs (Fernie et al. 2020; Rosado-Souza et al. 2023). Sugars enter the cytoplasm of SPC in the form of sucrose or hexoses. At this point, various reactions that are directly related to the cell's developmental stage can occur, which vary according to its position in the internode and age (Yanagui et al. 2022). Sucrose synthesis and accumulation are controlled by complex signalling cascades involving several enzymes, including three invertases, SuSy and SPS (Anur et al. 2020; Verma et al. 2019). Below, we will focus on these enzymes that regulate sugar storage and their utilization in the internode.

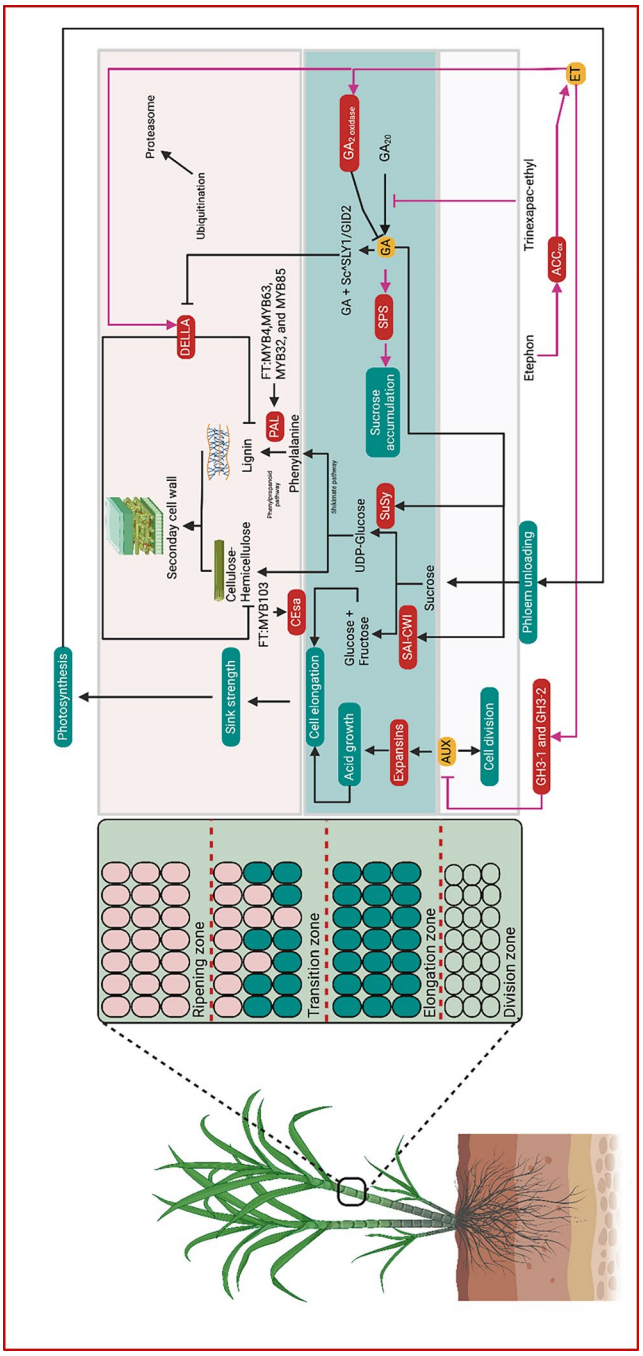

Fig. 9.4 The diagram illustrates the zones of the sugarcane internode and the key processes occurring in each zone. Oval shapes represent parenchyma cells. Cells without fill are in the division stage, green cells are without secondary cell wall formation and pink cells have secondary cell wall formation. Green rectangles indicate macro processes related to the preceding compound. Red rectangles highlight key proteins in each process. Yellow rectangles indicate the hormones involved. Solid lines represent upregulation, while truncated lines show downregulation. Black lines indicate processes occurring during the rapid growth stage and pink lines indicate processes during the ripening stage. Abbreviations used: *GA* gibberellin, *AUX* auxin, *ET* ethylene, *ABA* abscisic acid, *TF* transcription factor, *CesA* cellulose synthase, *SAI* soluble acid invertase, *CWI* cell wall invertase, *PAL* phenylalanine ammonia-lyase enzyme. (Created with BioRender.com)

9.6.1 Sucrose Synthase (SuSy)

SuSy activity depends on UDP, inorganic phosphate, two glutamate residues and one phenylalanine residue, which are essential for its function (Huang et al. 2016). In sugarcane, six genes encoding SuSy have been identified, named ScSuSy1, 2, 3, 4, 5 and 6 (Saavedra-Díaz et al. 2024; Zhang et al. 2013). In the transition and elongation internodes zones, SuSy shows notable activity, performing a reversible reaction, converting sucrose to UDP-glucose and fructose. UDP-glucose is an essential precursor and substrate for the synthesis of polysaccharides related to cell wall formation, starch, callose, or pectins (Botha 2018; Chen 2021; Stein and Granot 2019; Verma et al. 2011). Therefore, SuSy expression exhibits a gradient throughout the stem. In immature internodes, SuSy expression is high, decreasing in the middle zone of the stem and becoming low in the basal or mature internodes. This pattern is mainly observed in low-sucrose varieties or apical or immature internodes (Thirugnanasambandam et al. 2019; Verma et al. 2011).

It is believed that the low sucrose content in immature internodes and low-sucrose varieties is due to competition for UDP-glucose generated during cellulose synthesis, which is mediated by CesA proteins. Among these proteins, ShCesA1, 7 and 9 show high expression in elongating internodes. This is crucial, as cell wall formation is necessary for internode elongation (Casu et al. 2015; Wang et al. 2013). Therefore, sucrose levels are low during the stem elongation stage and increase as SuSy activity diminishes along with growth, i.e., during the ripening stage (Verma et al. 2019).

Considering the above, SuSy is seen as a generator of sink strength during the rapid growth stage. Here, GA signalling elevates the relative expression of SuSy between 50 and 80 days after exogenous application, consuming carbohydrates to enlarge internode volume and source strength (Nguyen et al. 2019; Rai et al. 2017; Verma et al. 2017). During the ripening stage, an increase in SuSy expression in response to ET has been observed, contributing to sucrose synthesis from reducing sugars (Chen et al. 2019a, b, 2024; Cunha et al. 2017; Neris et al. 2022; Guo et al. 2023). Nonetheless, SuSy6 has shown a negative correlation with sucrose accumulation under high soil moisture conditions, possibly favouring the reaction synthesising UDP-glucose, subsequently used in structural carbohydrate synthesis (Saavedra-Díaz et al. 2024). This behaviour is similar to that observed in rice, where basal ABA levels enhance SuSy activity, generating greater sink strength and reducing feedback on photosynthesis during the grain-filling stage (Tang et al. 2009). Thus, prevailing climatic conditions may influence SuSy's role during ripening.

9.6.2 Sucrose Phosphate Synthase (SPS)

SPS is considered the key enzyme in the sucrose biosynthesis pathway in sugarcane (Almeida-Silva and Maitto 2012). Two isoforms of SPS have been identified in sugarcane: SoSPS1, which is expressed in leaves, and SoSPS2, which shows constitutive expression throughout the plant (Sugiharto et al. 1997). Overexpression of SoSPS1 increases SPS and SAI activity, raising sucrose concentration and reducing sugars in SPC (Anur et al. 2020). Although SPS and SAI have opposing functions, a balance must be maintained between them, with SPS activity being higher than SAI to ensure a constant flow of hexoses and sucrose synthesis, stimulating sink strength and preventing feedback on photosynthesis (Mulyatama et al. 2022). For this reason, sugarcane varieties that show higher sucrose accumulation are characterized by high SPS activity in the upper third stem (Grof et al. 2007; Niazi et al. 2023).

SPS expression has been linked to signalling pathways regulated by GA and ET. In the case of GA, about 180 days after exogenous GA application, SPS expression in stems rises in the middle and upper third internodes, resulting in a significant rise in sucrose content (Nguyen et al. 2019; Rai et al. 2017; Verma et al. 2017). This is due to the enlarged internode volume and the generation of sink strength caused by invertase activity during the rapid growth stage. However, in response to ET during the ripening stage, sucrose synthesis via SPS in leaves is upregulated, enhancing source strength (Chen et al. 2019a, b; Cunha et al. 2017; Neris et al. 2022; Joshi et al. 2022). This is possibly regulated by the TF ERF12 and ERF112 (Saavedra-Díaz et al. 2024). However, ET response also triggers a significant increase in ABA synthesis in apical internodes, attributed to the upregulation of ABA synthesis enzymes, such as 1-Deoxy-d-xylulose 5-phosphate synthase (DXS) and 9-Cis-epoxycarotenoid dioxygenase9 (NCED9), along with ABA- 8′-hydroxylase1 (CYP707A1), suggesting the maintenance of ABA homeostasis (Cunha et al. 2017; Xu et al. 2022; Wu et al. 2022). This rise in ABA concentration also indicates that this hormone may regulate SPS expression, which could explain the increase in brix degrees in sugarcane sprayed with ABA (10 PPM) during the ripening stage (Hayamichi 1997). Although the effect of water deficit on sucrose accumulation is well known, the role of ABA and the ABA-ET relationship in sucrose accumulation remains unclear.

9.6.3 Invertases

In sugarcane, it is estimated that approximately 70% of the sucrose transported towards the internodes is converted into reducing sugars before being stored (Chandra et al. 2015; Verma et al. 2017). This process is primarily carried out by three types of invertases: neutral invertases (NI) in the cytoplasm, CWI in the cell wall space, and SAI in the vacuole (Wang et al. 2013). This sucrose hydrolysis

provides the necessary energy for growth, respiration and maintenance processes (Ansari et al. 2013).

Various studies have revealed that invertases show their maximum expression during the rapid growth stage and decrease in activity as ripening occurs. Additionally, these enzymes are more active in the apical internodes compared to the basal internodes (Chandra et al. 2015; Mason et al. 2022; Pan et al. 2009; Verma et al. 2011, 2017, 2019). Among them, NI has shown a positive correlation with early ripening under high soil moisture conditions in genome-wide association studies (GWAS), possibly associated with increased sink strength during the rapid growth stage, which favours early sucrose accumulation (Saavedra-Díaz et al. 2024). Nevertheless, the role of NI in sucrose accumulation has not yet been fully elucidated due to its irregular expression levels, possibly resulting from competition with SuSy for the substrate in the cytoplasm (Verma et al. 2019).

During the rapid growth stage, in response to GA, the expression of SAI and CWI increases, consuming sucrose for growth respiration (Nguyen et al. 2019; Rai et al. 2017; Verma et al. 2017). However, during the ripening stage, ET boosts the expression of NI and CWI in the internodes, which is associated with generating sink strength to reduce feedback on photosynthesis (Chen et al. 2019a, b; Cunha et al. 2017; Neris et al. 2022). Despite that, in corn, ABA basal concentrations (5–10 μM) have been observed to elevate CWI and SAI expression (Zhang et al. 2017). Considering the relationship between ABA and ET described earlier, the exact level of regulation of each hormone on invertases remains unclear. The expression of these invertases may have practical applications, such as in selecting sugarcane varieties, as high expression of CWI and SAI during the rapid growth stage has been associated with high sucrose varieties (Chandra et al. 2015). Figure 9.5 illustrates the hydrolysis processes and movement of sugar between the SE-CC complex and the SPC, involving the mentioned enzymes.

9.7 Conclusion and Future Perspectives

Understanding the source–sink relationship is crucial for developing genetic improvement or agronomic management strategies that lead to boosted crop yields. In grasses, phytohormones such as ET, GA, ABA, AUX, CK and carbohydrate concentration regulation, specifically through trehalose metabolism, play a pivotal role in regulating source activity or sink strength (Fig. 9.6). Generally, AUX and GA play their most significant role in the formation of sink organs and, consequently, in generating sink strength. Meanwhile, CK acts more in regulating leaf senescence, which can be caused by abiotic stress, feedback exerted by sucrose, trehalose metabolism and SIRT. Finally, ABA and ET have been associated with essential signalling pathways during the ripening stage, as they downregulate growth and upregulate proteins related to phloem loading and unloading, as well as enzymes involved in sucrose synthesis.

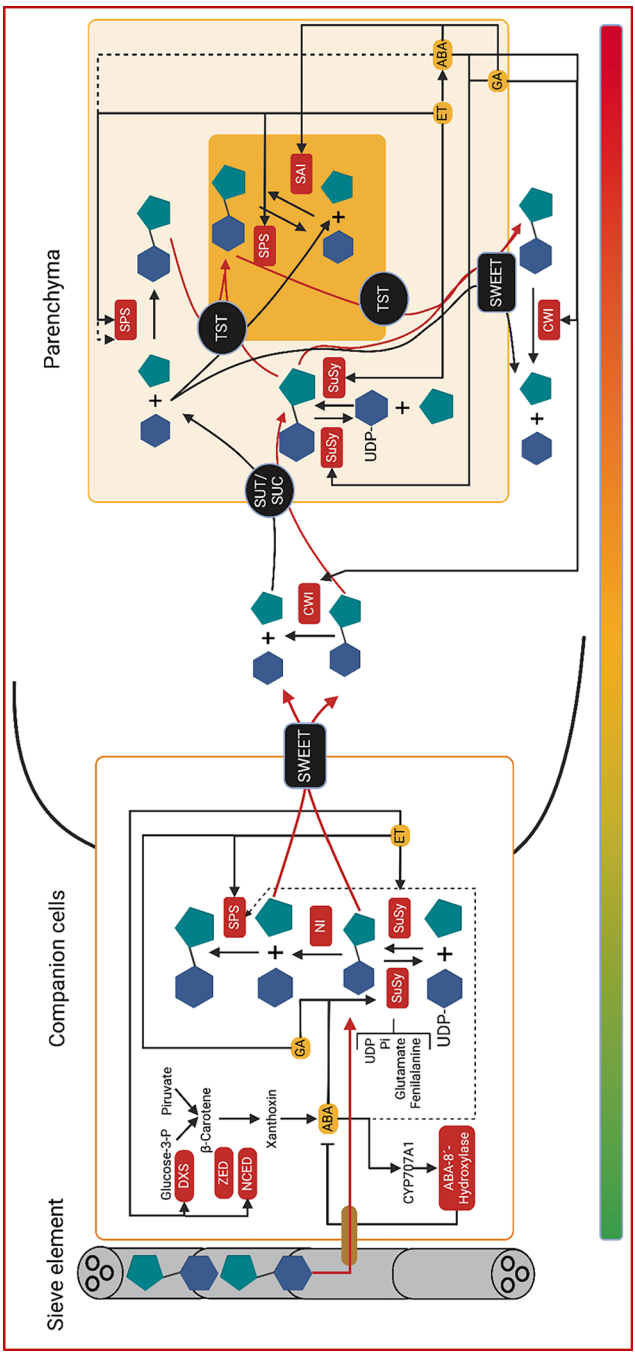

Fig. 9.5 The diagram illustrates the process of sucrose utilization and storage in sugarcane internode cells. Black and red arrows represent the movement of reducing sugars and sucrose through SWEET, SUT/SUC and TST transporters. Hexagons and pentagons together represent a sucrose molecule. The dark blue hexagon represents a glucose molecule, while the green pentagon indicates a fructose molecule. Brown rectangles represent intercellular connections via plasmodesmata, and the solid black line around the companion cell represents a suberin layer. Red rectangles highlight key proteins in each process, while yellow rectangles indicate the hormones involved. Black rectangles refer to sugar transporters. Solid lines represent upregulation; truncated lines show downregulation and dotted lines indicate potential regulations. The colour gradient at the bottom suggests sucrose concentrations, with red representing the highest level and green the lowest. Abbreviations: *NI* (neutral invertase), *CWI* (cell wall invertase), *SAI* (soluble acid invertase), *SuSy* (sucrose synthase), *SPS* (sucrose phosphate synthase), *GA* (gibberellin), *ET* (ethylene), *ABA* (abscisic acid), *ZED* (zeaxanthin epoxidase), *DXS* (1-deoxy-d-xylulose 5-phosphate synthase), *NCED* (9-cis-epoxycarotenoid dioxygenase9). (Created with BioRender.com)

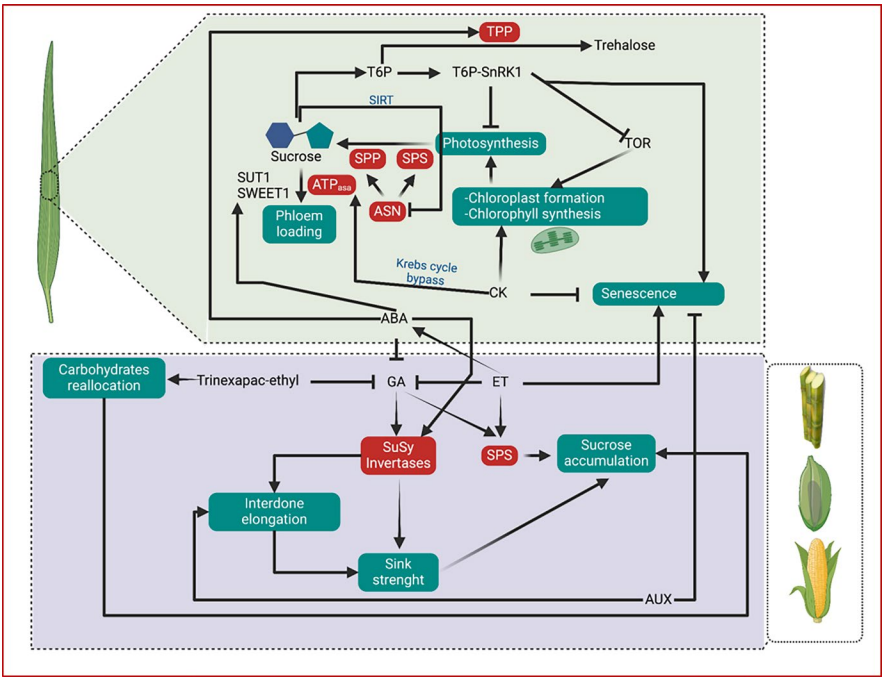

Fig. 9.6 Signalling model for regulating the source–sink relationship exerted by intrinsic factors in sugarcane, rice and corn. In the diagram, black arrows represent upregulation and truncated lines represent downregulation. Red rectangles refer to enzymes involved in each process. Green rectangles indicate macro processes related to the preceding compound. Here are the abbreviations and their meanings: *ET* ethylene, *ABA* abscisic acid, *GA* gibberellins, *CK* cytokinins, *AUX* auxins, *SuSy* sucrose synthase, *CWI* cell wall invertase, *SPS* sucrose phosphate synthase, *SPP* sucrose phosphate phosphatase, *SUT* carbohydrate antiporter transporter, *SWEET* carbohydrate transporter, *T6P* trehalose-6-phosphate, *TPS* trehalose phosphate synthase, *TPP* trehalose phosphate phosphatase, *SnRK1* sucrose non-fermenting-1 related kinases, *ASN* asparagine synthase, *TOR* target of rapamycin, *SIRT* sucrose-induced regulation of translation. (Created with BioRender.com)

The source–sink relationship in crops such as rice and corn has been extensively studied; nevertheless, advancements in regard to sugarcane remain limited. For this reason, studies are needed to integrate the balance between carbohydrate production in the leaves and sugar utilization in growth and accumulation in sink organs and how this can be regulated to raise productivity. These studies would lay the groundwork for subsequent genetic manipulation of molecular signalling related to the source–sink relationship or implementing management practices involving growth regulators that act throughout this relationship. The goal is to advance sucrose production per hectare, which appears to have plateaued in several agro-industries in recent years, posing a risk to sugarcane-producing countries' food and energy security.

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Chapter 10
MicroRNAs and their Role in Breeding Programs for Sugarcane Improvement

Eid M. Mehareb

Abstract Sugarcane miRNAs play several regulatory roles, including abiotic stress responses, disease resistance, and metabolic control. Understanding these roles provides insights into sugarcane biology and opens avenues for improving sugarcane cultivars through biotechnological approaches to enhance stress tolerance and yield. Additional research is required to fully elucidate the specific miRNAs involved and also their mechanisms of action in this economically important crop. These findings emphasize the significance of miRNA-mediated control in the drought response of sugarcane, a key bioenergy crop, and provide valuable insights for developing drought-tolerant sugarcane cultivars.

Keywords Sugarcane · miRNA · Abiotic stress · Drought · Disease · Metabolic regulation

10.1 Introduction

Sugar production is a significant agricultural sector worldwide, with approximately 80% of sugar derived from sugar cane and 20% from sugar beets. Various factors influence the global sugar economy, including climatic conditions, technological advancements, and market demand (Verma et al. 2024a, b; Li et al. 2024; Chen et al. 2024). Global sugar production has experienced significant fluctuations and growth in recent years, with 2022/2023 marking a record output of 177.3 million metric tonnes of raw value, and current forecasts project a 1.2% reduction for the 2023–2024 cropping season (https://foodinstitute.com/focus/sugar-production-isnt-looking-so-sweet/). Figure 10.1 outlines the major sugar-producing countries, their respective

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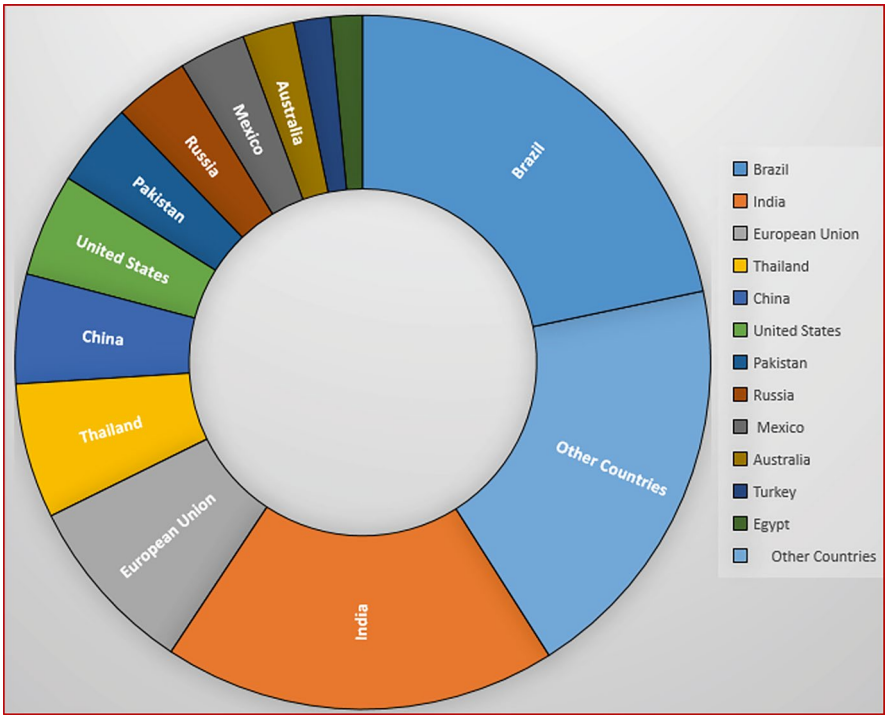

Fig. 10.1 Depicts the major sugar-producing countries, their relative production quantities, and their percentage contributions to total global output

production volumes, and their contributions as a percentage of the total global production.

10.2 Sugar Accumulation

Sugarcane's major product, sucrose, accumulates in the stalk internodes. Sucrose accumulation is fascinating in sugarcane (*Saccharum* spp.) as it produces about 75% of the world's sucrose. It is a dynamic continuous cleavage and synthesis process in sugar-storage parenchyma tissue. However, in sugarcane, the biochemical processes that control sucrose accumulation are still poorly understood and are the subject of intensive research to improve yield further. Sucrose synthase (SuSy, UDP-glucose: D-fructose 2-a-D-glucosyltransferase, EC 2.4.1.13) regulates sucrose accumulation and partitioning by converting sucrose and UDP into UDP-glucose and fructose in a reversible process. There is less knowledge on the sugarcane SuSy gene family members, and no research has been conducted to determine their allelic diversity in *Saccharum* species.

Although much effort has been put into molecular approaches to break through the apparent barrier for sucrose concentration in sugarcane stalks, the molecular mechanisms and genetics that restrict sucrose accumulation remain unknown. Our findings show that restraining expansive growth with water stress improves sucrose accumulation in both low- and high-sucrose clones. Sucrose accumulation was primarily described (72%) by an equation including photosynthesis, plant extension rate (PER), and plant number. The current study sought to test if this simple model holds true when temperature, rather than water stress, perturbs the source–sink balance. A thinning treatment was used to test the idea inherent in this equation that SC will increase if rivalry between plants for photoassimilation is minimized (Inman-Bamber et al. 2010).

The capacity of sugarcane to store large concentrations of sucrose in its culm necessitates adaptation to sustain cellular function under high solute loads. Examine the expression of 51 genes involved in abiotic stress to identify their role in sucrose accumulation by examining mature and immature culm internodes of a high-sucrose accumulating sugarcane variety. Expression of a subset of eight genes was investigated in mature internode tissues of sugarcane varieties, as well as ancestral and more broadly related species with varying sucrose concentrations. To assess the effects of sucrose buildup and drought stress, the expression of these genes was examined in internode tissue from a high-sucrose variety subjected to drought stress. Correlation analysis revealed a subset of stress-related genes that may be associated with sucrose accumulation in sugarcane culms, including genes encoding amino acid metabolism enzymes, a sugar transporter, and a transcription factor (Begcy and Dresselhaus 2018). Subsequent study of the expression of these stress-response genes in sugarcane under drought stress revealed a transcriptional profile distinct from that associated with sucrose buildup.

Genes with similarity to late embryogenesis abundant-related proteins and dehydrin, for example, were substantially activated in the presence of a drought, although this did not correlate with sucrose concentration. The expression of genes encoding proline biosynthesis was linked to both sucrose buildup and drought. Nonetheless, amino acid analysis revealed that proline was negatively correlated with sucrose concentration, and while total amino acid concentrations increased approximately sevenfold under drought, proline's relatively low concentration suggested that it played no osmoprotective role in sugarcane culms. While there was a shift in stress-related gene expression linked with sucrose buildup, distinct systems responded to the stress caused by water deprivation because different genes had changed expression (Iskandar et al. 2011).

Papini-Terzi et al. (2009) found significant overlap between genes related to sugar accumulation and water deficit adaptation, but less so with ABA treatment. The expression levels of the putative sugar transporter PST5 correlated positively with sucrose concentration throughout culm tissues and varieties. The PST5 sequence is similar to Arabidopsis sugar transporter-like proteins belonging to the ERD-6 (Early Responsive to Dehydration) transporter family (Kiyosue et al. 1998;

Johnson et al. 2006). Drought treatment elevated the ERD genes in Arabidopsis, and ERD-6 responded to both drought and cold. Drought in sugarcane found to modestly trigger the PST5 gene. The PST5-encoded transporter has recently been localized to the tonoplast and may be involved in sugar remobilization from the vacuole (Pioch 2010). Sugar transport is an important component of current models for sugar accumulation in sugarcane culm tissue (Rae et al. 2005), so the correlation of PST5 gene expression with sucrose content should pique interest in further functional analysis of this transporter's potential rate-limiting role in the sugar accumulation process.

10.3 The Functional Role of miRNAs

Few studies have focused on the tiny RNA population and its activities. To identify miRNA families and other small RNAs that may be involved in the sugarcane response to drought stress, small RNA libraries were created from control and drought-treated sugarcane ROC22 cultivars, and small RNA expression was profiled using Solexa sequencing technology (Genome Analyser IIx, Illumina). Putative target genes were predicted, and their activities were studied using web-based bioinformatics data. The expression profile of miRNA families synthesized by drought-resistant cultivar ROC22 leaves was determined after adding PEG to the growing substrate. There were 23 preserved miRNA families and 34 novel miRNA families found, as well as 438 potential target genes from 44 miRNA families characterized. The expression analysis indicated that 11 miRNA families were expressed differently in control and drought-exposed plants.

Genes linked to plant development and the 11 miRNA families identified stress tolerance, including SPBP, MYB, the AGO1-like gene, NCBP, BCP, CPI, and LSG, as possible targets. The other genes were down-regulated in response to drought stress, with the exception of SPBP, NCBP, and BCP. The initial discovery of 11 significant miRNA families that ROC22 and its targets express raises the possibility that they control the body's reaction to drought stress and serve as a foundation for further research. According to Lin et al. (2014), the other newly discovered miRNA families and their targets could have unique impacts on a number of metabolic pathways related to the growth and development of sugarcane. Abiotic stressors, including salt and drought, are important agricultural productivity barriers that cause large yield losses every year (Begcy and Dresselhaus 2018).

For plant breeders, maintaining plant output in unfavorable conditions is an ongoing issue. Drought continues to threaten our capacity to satisfy the demands of a growing global population as agriculture is being forced to marginal lands and the freshwater supply fluctuates due to unpredictable weather patterns. Due to the use of subpar water, irrigation is a consequence of soil salinization in a number of areas (Parihar et al. 2015; Verma et al. 2024c). Sugarcane agriculture has spread to areas with greater temperatures and less water resources, where drought is widespread, as a result of the rising need for food and renewable energy sources. For plant

breeders, maintaining plant output in unfavorable conditions is an ongoing issue. Additionally, in certain places, irrigation has led to soil salinization, which hinders sugarcane growth. The creation of better cultivars is made more difficult by the diverse range of processes used by plants to sustain yield production in various stressful situations, as well as the low heritability of such characteristics. However, developments in allied fields like genetics, physiology, and molecular biology have greatly advanced our understanding of how plants react to stress (Gao et al. 2007; Perez-Clemente et al. 2013; Begcy et al. 2019). Growing research indicates that a number of genes may play a part in lessening the constraints that environmental stressors place on crops (Umezawa et al. 2006; Begcy et al. 2012; Li et al. 2015; Fujita et al. 2013; Begcy and Walia 2015; Chen et al. 2016). According to Luhua et al. (2013), genes that encode proteins with uncertain activities are intriguing sources for learning about novel plant characteristics. The majority of proteins in most genome projects are unknown genes, accounting for 30% of rice proteins (Yu et al. 2002), and 33% of maize proteins (Schnable et al. 2009). Twenty-seven sugarcane cDNA libraries from various tissues of four cultivars that were subjected to a range of conditions, including low and high temperatures and endophytic nitrogen-fixing bacterial interactions, among others, during various developmental stages, yielded 237,954 ESTs (expressed sequence tags) in sugarcane. Of the 43,141 putative and distinct sugarcane transcripts that were constructed from the EST collection (Vettore et al. 2003), 38% encoded proteins with unidentified activities (Vettore et al. 2001).

In a prior work, we found that when sugarcane plants were subjected to diverse biotic and abiotic stressors, 179 genes were differently expressed (Rocha et al. 2007). It is interesting to note that drought altered the expression of 93 genes in sugarcane, causing the most alterations in the transcriptome. One of these genes, Scdr1 (Sugarcane drought-related-1), has been demonstrated to improve tolerance to environmental stressors, whereas other genes encode proteins with unknown roles (Begcy et al. 2012). Here, we have functionally described the activity of *Scdr2*, a little gene that codes for a protein that is triggered by drought. Four cultivars of sugarcane with varying levels of drought tolerance were tested for the *Scdr2* transcriptional expression pattern when exposed to drought. When subjected to drought and salt, transgenic tobacco plants overexpressing *Scdr2* showed increased levels of photosynthesis, stomatal conductance, transpiration rates, and internal CO_2 concentrations compared to wild-type (WT) plants. Furthermore, the transgenic *Scdr2* plants showed increased biomass under these circumstances.

10.3.1 Invertases

Hexose or sucrose transporters carry sucrose from the apoplast into sugarcane sink cells. Hexose transporters may use the breakdown products of fructose, sucrose, and glucose that are produced when the enzymes known as invertases (-Fructofuranosidase) cleave the sugar. Many genes in plants encode invertases; for instance, rice has

19 and Arabidopsis has 17 (Ji et al. 2005; Conradie 2011). The number of genes in sugarcane is still unknown. Enzymes that fall into one of three isoform categories are often encoded by the genes. According to Grof and Campbell (2001), they are distinguished by their subcellular localization, solubility, and pH optima. With a pH optimum of 4.5–5, acid invertases can be classified as either soluble or insoluble (Sturm 1999).

Cell wall invertase, often referred to as insoluble invertase, is attached to the cell wall with its catalytic subunit oriented toward the apoplast. It breaks down apoplastic sucrose into components that hexose transporters may use (Xu et al. 1996). Apoplastic invertases have been shown to have a major impact on the sink–source relationship in a variety of animals. Numerous aspects of the function of yeast acid invertases (YI) and their contribution to photoassimilate transport were clarified by the expression of YI in several plant species (Stitt et al. 1991; Sonnewald et al. 1991; Heineke et al. 1992; Sonnewald et al. 1997). Although the impacts varied by species, they all typically had a negative impact on the plants, highlighting the role that invertases play in the sink tissues' carbon partitioning.

Vacuolar acid invertase (VAI) is the name of the soluble acid isoform that is found in the vacuole (Leigh et al. 1979; (Fig. 10.2). When sugarcane internodes are young, VAI is quite active; however, as the internodes get older, this activity decreases (Zhu et al. 2000; Rae et al. 2011). In suspension cultures and the young

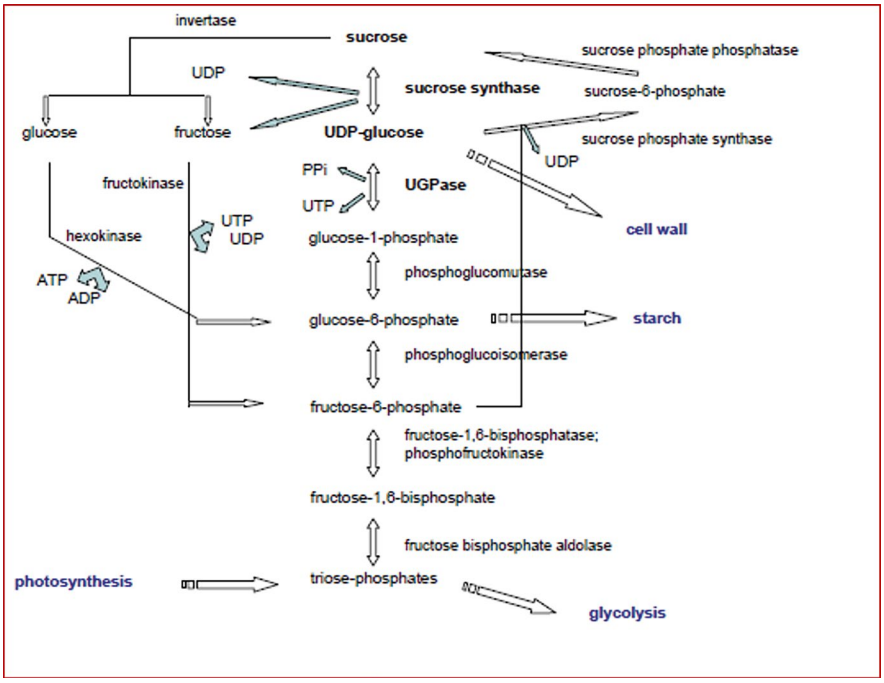

Fig. 10.2 Mechanism of sucrose metabolism

internodes of glasshouse plants, NI suppression in sugarcane using an antisense construct results in reduced activity, elevated sucrose contents, and enhanced SuSy activity (Joubert 2006; Rossouw et al. 2007). Although there was less of a decrease in NI activity in plants than in suspension cultures, decreased growth rates were associated with higher sucrose levels and SuSy activity (Joubert 2006). Additionally, as internodes grew older, NI activity declined even more. Growth rates and glucose and fructose concentrations dropped in glasshouse plants and suspension cultures (Rossouw et al. 2007). Because there were less hexoses available for respiration and growth, transgenic plants had decreased vitality. The enhanced SuSy activity most likely took up the majority of sucrose hydrolysis and cycling in order to make up for the decreased NI activity.

10.3.2 Sugar Accumulation

Sugar accumulation in sugarcane plants is a difficult process influenced by various factors, including plant growth regulators, genetic expression, and source–sink dynamics. Exogenous treatments, such as glyphosate and gibberellic acid (GA_3), have been shown to enhance sucrose content by increasing sink strength and altering source–sink communication. Glyphosate treatment significantly increased sucrose content in immature internodal tissues, while GA_3 treatment improved sink capacity and modified sugar distribution within the plant (Dill Jr 1981; Singh and Chandra 2021). Furthermore, controlling sucrose buildup depends critically on the expression of important genes involved in sucrose metabolism, including invertases and sucrose phosphate synthase (SPS). These genes respond to sink demand, particularly pronounced in high-sucrose varieties (Verma et al. 2019; Chen et al. 2019). Additionally, the dynamics of tillering and the timing of tiller mortality significantly influence early sugar accumulation, highlighting the importance of genetic traits in optimizing sucrose yield (Saez et al. 2019). Overall, enhancing sucrose accumulation in sugarcane requires a multifaceted approach considering genetic and environmental influences.

10.4 MicroRNAs and Their Role in Breeding Programs for Sugarcane Improvement

MicroRNAs play an essential role in improving sugarcane (*Saccharum* spp.) through their regulatory functions in response to several abiotic stresses and metabolic processes. Research indicates that miRNAs, such as miR395, miR160, and miR167, are involved in mechanisms that confer tolerance to aluminum stress, enhancing root formation and detoxification processes in specific sugarcane genotypes (de Oliveira Leite Silva et al. 2021). Furthermore, miRNAs regulate characters such as

Water shortage tolerance, disease resistance, and sugar metabolism, although specific miRNAs linked to sugar accumulation in sugarcane remain unidentified (Swapna and Kumar 2017). Advanced biotechnological approaches, including RNA interference (RNAi) and CRISPR/Cas systems, can be employed to manipulate miRNA expression or objective genes, helping the development of sugarcane cultivars with desirable characters (Teotia et al. 2020).Overall, incorporating miRNA research into breeding programs has tremendous promise for boosting sugarcane resilience and production in the face of environmental problems.

The Functional Role of miRNA in Sugarcane
Since they affect a number of physiological functions and reactions to external stressors, microRNAs (miRNAs) are essential regulators in sugarcane. Through target cleavage and translational suppression, these little non-coding RNA molecules, which are usually 20–24 nucleotides long—play a key role in the post-transcriptional control of gene expression.

10.4.1 Key Functional Roles of miRNAs in Sugarcane

10.4.1.1 Regulation of Stress Responses

The way sugarcane reacts to abiotic stressors including drought, cold, and nutrient shortages is strongly influenced by miRNAs:

- *Drought Tolerance*: Specific miRNAs like miR164, miR166, and miR398 have been linked to drought response mechanisms, regulating genes associated with stress tolerance and metabolic pathways (Swapna and Kumar 2017; Yang et al. 2017).
- *Cold Stress*: Studies have identified several miRNAs that exhibit differential expression under freezing conditions, suggesting their role in enhancing cold tolerance. Notably, miR156 has increased expression during cold stress, contributing to improved growth and stress resilience (Yang et al. 2017).
- *Nutrient Deficiency*: miRNAs for example miR156 and miR171 are involved in response to low potassium (K^+) conditions, regulating root formation and signaling pathways related to nutrient uptake (Nandakumar et al. 2021; Zhang et al. 2022).

10.4.1.2 The Contribution of miRNAs to Drought Tolerance in Sugarcane

miRNAs significantly contribute to drought tolerance in sugarcane by regulating gene expression and modulating various physiological responses to drought stress. Here are the key mechanisms through which miRNAs facilitate drought tolerance in sugarcane:

- *Differential Expression of miRNAs*

- Research has identified numerous miRNAs that exhibit differential expression in response to water stress. For instance, studies have exposed that specific miRNAs are upregulated or downregulated depending on the drought tolerance of the sugarcane cultivar. In one study, 18 miRNA families were identified in drought-tolerant and drought-sensitive varieties, with several miRNAs differentially expressed during drought conditions, indicating their role in adaptive responses to water shortage (Gentile et al. 2015; Selvi et al. 2021).
- *Targeting Key Regulatory Genes*
- miRNAs target various genes involved in critical pathways related to drought response. For example, they regulate transcription factors, signaling proteins, and enzymes crucial for stress tolerance. Target prediction analyses have revealed that many miRNAs are associated with genes encoding proteins included in signal transduction, oxidative stress tolerance, and carbohydrate metabolism, which are vital for maintaining plant health under drought stress (Gentile et al. 2015).
- *Regulation of Physiological Processes*
- MiRNAs influence several physiological processes that enhance drought tolerance, including:

  Leaf Senescence: Certain miRNAs are involved in regulating leaf senescence, which, in times of drought, may be an adaptive reaction to preserve resources (Ferreira et al. 2012).
  Root Development: miRNAs also play a role in root architecture, promoting deeper root systems that can access water more effectively during periods of drought.

- *Complex Regulatory Networks*
- The interaction of miRNAs with their targets creates complex regulatory networks that help sugarcane plants adjust to varying drought conditions. For instance, some miRNAs were found to have opposing expression patterns in different cultivars, suggesting a sophisticated level of regulation that allows sugarcane to fine-tune their responses based on genetic background and environmental conditions (Ferreira et al. 2012).
- *Biotechnological Applications*

- Understanding the role of miRNAs in water lack tolerance opens avenues for biotechnological applications, such as developing transgenic sugarcane varieties with enhanced drought resistance. By manipulating the expression of specific miRNAs, it may be possible to improve the resilience of sugarcane crops to drought stress, thereby ensuring better yield and sustainability in water-limited environments.

10.5 The Specific miRNAs Are Consistently Upregulated Across Different Drought-Tolerant Sugarcane Varieties

Drought stress significantly affects sugarcane productivity, and specific miRNAs have been identified as key regulators in drought-tolerant varieties. Research consistently highlights several miRNAs that are upregulated across different drought-tolerant sugarcane cultivars (Ferreira et al. 2012).

10.5.1 Key Upregulated Upregulated miRNAs

- *ssp-miR164*: identified as being involved in stress tolerance pathways and exhibiting differential expression in response to drought.
- *ssp-miR394*: This miRNA is also upregulated during drought stress, showing its involvement in water deficit response pathways.
- *ssp-miR397*: Found to be suggestively expressed under drought conditions, suggesting its role in regulating stress-related genes.
- *ssp-miR399*: This miRNA was validated for its expression during drought, contributing to understanding drought tolerance.
- *miR528*: Another miRNA showed increased expression levels under drought stress, showing its potential role in conditions adaptation.

10.5.2 Broader Insights

Together, the investigations highlight the intricate regulatory networks, including miRNAs in the drought response of sugarcane. While specific miRNAs are consistently upregulated, the interplay between these miRNAs and their target genes, such as stress response-related proteins and transcription factors, remains an area for further exploration. Understanding these interactions could enhance breeding strategies for drought resilience in sugarcane.

10.5.3 The Role miRNAs Play in Drought Tolerance in Sugarcane

MicroRNAs play a key role in enhancing drought tolerance in sugarcane by regulating gene expression involved in stress responses. These small non-coding RNAs modulate various physiological and biochemical pathways, enabling plants to adapt to water scarcity.

10.5.3.1 Mechanisms of miRNA Action

- miRNAs target mRNAs linked to drought stress responses and post-transcriptionally control gene expression., such as transcription factors and signaling proteins (Liang et al. 2024a, b; Selvi et al. 2021).
- Specific miRNAs, like Osa-miR393 and miR166, have been linked to improved osmotic stress tolerance and antioxidant defense mechanisms (Singh et al. 2022).

10.5.3.2 Differential Expression in Varieties

- The research identified 1224 conserved miRNAs in sugarcane, with 145 being differentially expressed in drought-susceptible and tolerant varieties, indicating their potential in breeding programs (Selvi et al. 2021).
- The study also demonstrated how metabolic pathways, such the sulfur-relay system, play a role in drought response (Zou et al. 2023).

10.5.3.3 Future Implications

- Understanding miRNA functions can lead to biotechnological applications aimed at developing drought-resistant sugarcane varieties, crucial for sustainable agriculture in arid regions (Singh et al. 2022; Riyazuddin et al. 2023).

While miRNAs are pivotal in drought tolerance, the complexity of their interactions with other regulatory elements, such as long non-coding RNAs, suggests that a multifaceted approach is necessary for comprehensive drought resilience strategies.

10.6 The Regulation of miRNAs Gene Expression During Water Deficit Stress

MiRNAs are essential modulators of several physiological and biochemical processes that control gene expression in plants during drought stress. Their involvement in drought response mechanisms is multifaceted, influencing transcription factors, stress-associated genes, and hormonal signaling pathways.

10.6.1 Mechanisms of miRNA Action

- *Post-Transcriptional Regulation*: miRNAs primarily function by repressing translation or cleaving mRNA of target genes, which include transcription factors, such as MYB and ARF, crucial for drought response (Liang et al. 2024a, b; Kaur et al. 2024).
- *Diverse Targets*: Specific miRNAs, such as miR159, miR169, and miR397, have been recognized as significant regulators in various plant species, including rice and chickpeas, enhancing drought tolerance through modulation of stress-responsive genes (Kaur et al. 2024; Kumar et al. 2024).

10.6.2 Physiological and Biochemical Impacts

- *Stress Adaptation*: miRNAs influence processes like photosynthesis, respiration, and osmotic regulation, thereby improving plant resilience to water scarcity
- *Genotype Variation*: Differential expression of miRNAs under drought conditions has been observed across chickpea genotypes, indicating a complex regulatory network that varies with genetic background.

While miRNAs are pivotal in enhancing drought tolerance, challenges remain in fully understanding their regulatory networks and potential applications in crop improvement. Future research should focus on integrating miRNA-based strategies with traditional breeding to develop resilient plant varieties.

10.7 The Biological Functions of the Upregulated miRNAs in Drought-Tolerant Sugarcane

In drought-tolerant sugarcane cultivars, the increased microRNAs perform a number of vital biological roles that improve the plant's resistance to water stress. Here are the key biological functions associated with these miRNAs.

10.7.1 Regulation of Stress-Responsive Genes

Upregulated miRNAs target genes involved in stress response pathways, particularly those associated with drought tolerance. For example, miR159 targets MYB transcription factors, which are crucial for regulating stress-responsive gene expression and enhancing drought resilience by modulating physiological responses to water scarcity (Swapna and Kumar 2017; Selvi et al. 2021).

10.7.2 Modulation of Transcription Factors

miRNAs such as miR164, miR166, and miR171 regulate various transcription factors that mediate plant responses to drought. These transcription factors can influence processes such as leaf senescence, root development, and overall plant architecture, which are vital for maintaining function under drought conditions (Gentile et al. 2015; Swapna and Kumar 2017).

10.7.3 Oxidative Stress Management

MiRNAs, such as miR398 and miR396, are linked to regulating genes included in oxidative stress responses. These miRNAs help manage reactive oxygen species (ROS) levels during drought stress, protecting the plant from oxidative damage, and maintaining cellular integrity (Selvi et al. 2021).

10.7.4 Hormonal Signaling Pathways

The regulation of hormone signaling pathways, especially abscisic acid (ABA) signaling, which is essential for plants' responses to drought, is mediated by certain increased miRNAs. For instance, miR166 has been associated with ABA-dependent stress responses, helping the plant adjust its growth and development in response to water availability.

10.7.5 Regulation of Metabolic Pathways

miRNAs also influence metabolic pathways related to carbohydrate metabolism and transport, which are essential during drought conditions. They can regulate genes involved in sugar accumulation and transport, affecting the plant's energy status and overall resilience to stress (Gentile et al. 2015; Swapna and Kumar 2017).

10.7.6 Cross-Talk Between Pathways

The upregulated miRNAs facilitate cross-talk between various signaling pathways, integrating responses to drought stress with other physiological processes such as nutrient uptake and growth regulation. This interconnected regulatory network

allows sugarcane to adapt more effectively to changing environmental conditions (Gentile et al. 2015).

10.8 The Interaction of Regulated miRNAs with Other Regulatory Pathways in Drought-Tolerant Sugarcane

Upregulated microRNAs in drought-tolerant sugarcane interact with various regulatory pathways, contributing to the plant's ability to manage both water deficit stress and associated physiological processes. Here are the key interactions and functions of these miRNAs.

10.8.1 Cross-Talk with Transcription Factors

miRNAs, such as miR159, miR164, and miR171 target transcription factors involved in stress responses. For instance, miR159 targets the MYB transcription factor family, crucial in regulating stress-responsive genes. This interaction helps modulate gene expression that enhances drought tolerance and overall plant resilience under stress conditions (Lin et al. 2014; Selvi et al. 2021).

10.8.2 Regulation of Hormonal Signaling Pathways

Abscisic acid (ABA)-related hormonal signaling pathways, which are essential for drought response are influenced by miRNAs. Increased by influencing the expression of genes involved in the ABA signaling pathway, upregulated miRNAs can improve a plant's capacity to adapt to water constraint. According to Gentile et al. (2015), this modulation may result in physiological alterations, including stomatal closure, which lowers water loss during drought.

10.8.3 Modulation of Oxidative Stress Responses

Several upregulated miRNAs, including miR398, are involved in managing oxidative stress, which can be exacerbated during drought. These miRNAs regulate the expression of antioxidant enzymes and proteins that help mitigate oxidative damage, thus protecting the plant during periods of water stress. This interaction is essential for maintaining cellular integrity and function under drought conditions (Lin et al. 2014; Selvi et al. 2021).

10.8.4 Integration with Metabolic Pathways

miRNAs play a role in integrating various metabolic pathways related to carbohydrate metabolism and transport. By targeting genes involved in these pathways, miRNAs help optimize energy use and resource allocation during drought stress. This integration is essential for sustaining growth and development while coping with limited water availability (Gentile et al. 2015; Selvi et al. 2021).

10.8.5 Influence on Growth and Development

The expression of miRNAs can affect growth-related processes, such as leaf senescence, root development, and overall plant architecture. For example, miR164 and miR166 are involved in regulating genes that influence leaf senescence and root growth, which are critical for maximizing water uptake and minimizing water loss during drought (Lin et al. 2014; Gentile et al. 2015).

10.9 Complex Regulatory Networks

The interactions of miRNAs with their target genes create complex regulatory networks that allow sugarcane to fine-tune its responses to drought. These networks vary significantly depending on the cultivar, the drought stress applied, and the environmental conditions. This variability highlights the adaptability of miRNA-mediated regulation in response to changing stress conditions (Gentile et al. 2015; Selvi et al. 2021). MicroRNAs play a crucial role in regulating drought responses in sugarcane, an important biofuel crop. Studies have identified numerous miRNA families expressed in sugarcane leaves under drought stress, with several being differentially expressed (Ferreira et al. 2012; Lin et al. 2014; Liang et al. 2024a, b). These miRNAs target genes involved in plant growth, stress resistance, and various metabolic pathways (Zanca et al. 2010; Lin et al. 2014). MiRNA regulation and evolution in polyploid sugarcane can be better understood by comparing sugarcane and sorghum, which indicates that miRNAs and their targets are conserved (Zanca et al. 2010). Transcription factors, proteins linked to oxidative stress tolerance, cell remodeling, and hormone signaling are all involved in the intricate regulation of sugarcane miRNAs in response to drought. By using genetic markers and biotechnological techniques, it is possible to improve sugarcane's drought tolerance by comprehending this regulatory network (Ferreira et al. 2012; Gentile et al. 2015).

10.9.1 Disease Resistance

Additionally, miRNAs are essential for sugarcane's defense against infections. The relationship between sugarcane and the red rot disease *Colletotrichum falcatum* has been shown to include miRNAs. To improve resistance to fungal infections, some miRNAs control the expression of genes linked to stress responses, signaling pathways, and defense responses (Nandakumar et al. 2021).

10.9.2 Metabolic Regulation

Although direct links between miRNAs and sugar accumulation in sugarcane are still being explored, there is evidence suggesting that miRNAs may regulate metabolic pathways associated with sugar production. For example, although several miRNAs associated with sugar buildup in sugarcane have not been definitively identified, their roles in related crops like sorghum indicate a potential regulatory function in sugar metabolism (Swapna and Kumar 2017).

10.9.3 Developmental Processes

MiRNAs have a role in a number of sugarcane developmental processes, including axillary bud growth and overall plant architecture. The regulation of transcription factors by miRNAs influences these developmental traits, contributing to the plant's adaptability and productivity (Swapna and Kumar 2017).

10.10 Conclusion and Future Perspectives

miRNAs play diverse regulatory roles in sugarcane, including abiotic stress responses, disease resistance, and metabolic control (Fig. 10.3). Understanding these roles sheds light on sugarcane biology and offers possibilities for improving sugarcane cultivars through biotechnological treatments targeted at increasing stress tolerance and productivity. Additional study is required to thoroughly understand the particular miRNAs implicated and their modes of action in this commercially significant crop. Additionally, miRNAs play a critical role in regulating sugarcane's ability to withstand drought by influencing gene expression and physiological responses that help the plant deal with water stress.

Continued research into these regulatory mechanisms will enhance our understanding and ability to improve drought resilience in this important crop. Then, drought-tolerant sugarcane cultivars' increased miRNAs help them be more

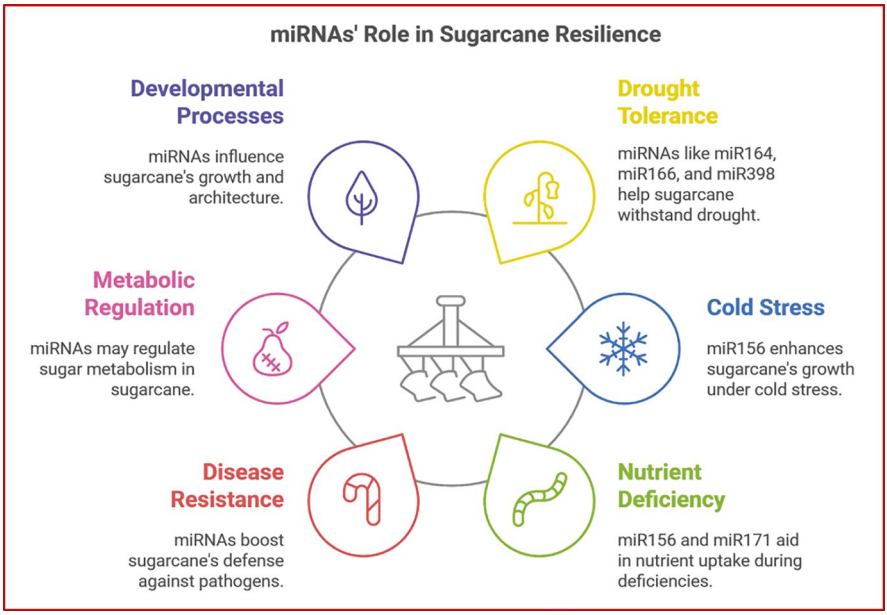

Fig. 10.3 The key functional roles of miRNAs in sugarcane plants

resilient to stress by controlling genes that respond to stress, altering transcription factors, oxidative stress management, hormonal signaling, and metabolic pathways. Knowing these roles can help breeders develop more resilient sugarcane to water stress and shed light on the molecular processes behind drought tolerance. Moreover, upregulated miRNAs in drought-tolerant sugarcane interact with multiple regulatory pathways, including transcription factors, hormonal signaling, oxidative stress responses, and metabolic pathways. These interactions enable the plant to effectively manage drought stress while maintaining growth and development, showcasing the intricate regulatory networks that miRNAs orchestrate in response to environmental challenges. Understanding these interactions further enhances the potential for developing improved sugarcane varieties through biotechnological approaches.

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Chapter 11
The Future Use of Molecular Markers in Sugarcane Breeding

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Abstract Sugarcane (*Saccharum* spp. hybrids) is a prominent cash crop that produces 78% of the global sugar production and is widely utilized for biofuel production. Due to its highly heterozygous genome, which has a high frequency of polymorphisms, and an extended breeding cycle that spans up to 10–15 years, typical breeding methods are problematic to use. Remarkably, all major producing countries in the world have selected and bred new varieties and continuously upgraded them as a solid guarantee to promote the steady development of the sugarcane industry. The key aspects of the selection process focus on traits such as high-sucrose content, strong resistance to diseases such as red rot, smut, brown rust, and leaf scald, low flowering propensity, and robust ratooning ability. The development and utilization of wild germplasm is very limited in the case of sugarcane; consequently, breeding technology in sugarcane cultivation is lagging compared to crops such as rice, maize, and soybeans. In this context, molecular markers have emerged as a transformative tool in modern plant breeding, offering unprecedented precision and efficiency in developing improved sugarcane varieties. The utilization of molecular markers was popularized in plant breeding in the era of breeding 3.0, along with techniques such as transgene, gene editing, and genomic selection to develop new varieties by selecting or aggregating the key regulating genes.

Molecular markers, including various types such as RFLP, SSR, and SNP, have transformed how breeders approach the genetic enhancement of crops. Molecular markers have played a crucial role in identifying genetic polymorphism linked with desirable agronomic traits, enabling precise and effective selection. Selection and

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planting of desired trait-bearing varieties, such as prevalent disease-resistant varieties are one of the cost-effective and practical strategies for developing sugarcane varieties with desired qualities. To enhance this process, molecular-assisted selection breeding is adopted, offering a reliable and effective method that is usually minimally affected by peripheral environmental factors. Recently, methods like high-throughput sequencing and genotyping have improved the capability to identify and use molecular markers. Diversity array technology (DArT), which uses a high-yield microarray platform to cover the whole genome and identify hundreds of thousands of polymorphic markers in a single study, could be a viable large-scale approach. Bulked segregant analysis (BSA) combined with molecular marker analysis of quantitative trait loci has proven effective in developing genetic maps around resistance genes in sugarcane, aiding in the fight against diseases and pests. Similarly, integrating omics data with molecular markers could offer even more significant advantages in the breeding field by predicting the breeding value of complex traits and thus enhancing the development of superior varieties. Despite these molecular markers incorporated in breeding approaches, they face several difficulties, such as high cost, resource requirements of molecular marker programs, and genome complexity. However, integrating artificial intelligence and machine learning will further boost the ability to elucidate complicated genetic information, driving the development of next-generation sugarcane varieties. The ongoing advancements in molecular marker technologies will ultimately determine the future of sugarcane breeding.

Keywords RFLP · SSR · SNP · BSA · DArT · Omics · Genome editing · *Saccharum* spp. hybrids

11.1 Introduction

Sugarcane (*Saccharum* spp. hybrids) is a prominent cash crop producing 80% of the global sugar production and widely utilized as a source of biofuel production (Verma et al. 2024a, b). Efforts were made to develop sugarcane varieties with desirable qualities like high-sucrose content and resistance to diseases, pests, and abiotic stresses that could produce satisfactory yields over several harvests through breeding programs (Verma et al. 2019a, b; Wu et al. 2022; Liang et al. 2024; Gujjar et al. 2024b, c). Plant breeding has developed through several stages that align with scientific knowledge and technical capacity advances (Li et al. 2024; Chen et al. 2024). In the beginning, breeders depended on genetics for selection; however, environmental factors frequently complicated their efforts, leading to phenotypes influenced by the combination of genotype (G), environment (E), and genotype × environment (G × E) effects (Kumari et al. 2024). Consequently, phenotypic selection was naturally biased by environmental influences. The discovery of DNA

structure revolutionized plant breeding, giving rise to the fields of genomics and molecular breeding. Molecular markers have become essential tools for DNA fingerprinting, characterization, diversity analysis, gene/QTL mapping, validation, and selection. Examples of these markers include RFLP, RAPD, ISSR, STS, SSR, SNPs, InDels, and microarrays. These markers made it possible to use advanced breeding methods such as marker-assisted selection (MAS), which selects multiple effective minor QTLs within an individual, pyramiding of major and minor QTLs into a single background, and introgression of major single genes or QTLs (Vishwakarma et al. 2022). Therefore, there have been four stages in the evolution of agricultural breeding: primitive domestication and selection (Breeding 1.0), traditional or conventional breeding (Breeding 2.0), molecular breeding (Breeding 3.0), and the newly emerging field of molecular design breeding, or smart breeding (Breeding 4.0), which is currently transitioning from theoretical frameworks to practical implications (Shen et al. 2022; Xu et al. 2022a, b).

Sugarcane breeding has a relatively short history, with the first interspecific crosses emerging in the early 1900s in Indonesia and India (Aitken 2022). During the late nineteenth and early twentieth centuries, sugarcane breeding entered what is often referred to as the "2.0 era," characterized by the adoption of crossbreeding strategies, quantitative genetics, and statistical approaches (Zhang et al. 2023a). However, sugarcane presents unique challenges for breeders. As an allopolyploid and aneuploid crop with a highly complex genetic background ($2n$ = 100–130, approximately 10 Gb), its hybrid progeny displays comprehensive segregation for traits (Piperidis et al. 2010). Moreover, the typical breeding cycle for sugarcane is notably long, ranging from 10 to 15 years (Kandel et al. 2018). The crop's large size (about 3–4 meters tall), long growing period (approximately 10–14 months), and perennial nature add further complexity to the phenotyping process, which is labor-intensive and time-consuming (Lu et al. 2024).

Despite these challenges, the development and utilization of wild germplasms remain limited, and breeding technologies have not advanced as rapidly as in other crops. However, using molecular markers associated with important agronomic traits offers a promising solution (Song et al. 2019; Singh et al. 2019; Huang et al. 2024; Lu et al. 2024). Marker-assisted selection (MAS) can be employed to select desirable varieties at the early stages of breeding programs and choose the best cross-parents. This approach could significantly reduce the time and cost required to develop new sugarcane varieties and assist in overcoming the existing barriers in sugarcane breeding (Lu et al. 2024).

11.2 Overview of Molecular Markers

Molecular markers are distinct variations in DNA nucleotide sequences that are closely linked to a target gene that controls a specific attribute in individual organisms or species (Amiteye 2021). These markers are usually located in proximity to the gene they are linked with, ensuring that they are inherited together with the gene

that expresses the trait (Collard et al. 2005). However, it is important to note that molecular markers do not influence the traits or biological functions associated with the linked gene or genes. Instead, their value lies in their tight association with specific traits or genes of interest (Amiteye 2021). This close association enables the identification of associated traits, even in cases where the exact genes involved are unknown or cannot be directly detected (Malviya et al. 2020; Chen et al. 2020; Reddy et al. 2021). This makes molecular markers a powerful tool in genetics and breeding programs, as they facilitate the identification and selection of desirable traits in organisms, thus aiding in the improvement of various species, including crops like sugarcane (Amiteye 2021; Guo et al. 2021; Xu et al. 2022a, b).

Numerous molecular markers have been developed, including single nucleotide polymorphism (SNP), microsatellite or simple sequence repeat (SSR), amplified fragment length polymorphism (AFLP), random amplification of polymorphic DNA (RAPD), and diversity arrays technology (DArT) markers (Gupta et al. 2001). Currently, molecular markers are extensively used in sugarcane breeding to enhance the efficiency and precision of selecting desirable traits. Employing molecular markers associated with specific characteristics, such as yield, sucrose content, and disease resistance, to detect and choose the most productive plants early in the breeding process is one of the main uses of marker-assisted selection (MAS). This approach is reliable and efficient, significantly reducing the traditionally long breeding cycle while being rarely affected by the external environment and allowing breeders to manage the vast genetic diversity within sugarcane populations more effectively (Lu et al. 2024). Moreover, dominant markers like AFLP, SSR, and DArT are extensively used for diversity analysis and genome-wide association mapping (GWAS) (Aitken 2022). Breeders can better understand the genetic relationships between various clones and varieties using molecular markers. This knowledge is essential to developing effective crossbreeding techniques (Table 11.1).

Different stresses, including abiotic and biotic stresses, significantly affect sugarcane productivity (Verma et al. 2020a, b, c; Kumari et al. 2022, 2023; Jagadesh et al. 2024). Various studies have revealed the potential of molecular markers to eliminate the drawbacks of conventional breeding methods since they possess a complex genome. Integrating molecular markers with traditional breeding methods and various emerging omics approaches paved the way for developing distinct stress-tolerant cultivars (Singh et al. 2020; Sharma et al. 2021, 2022; Kumar et al. 2023; Afghan et al. 2024).

11.2.1 Abiotic Stress

Abiotic stresses affecting the yielding capacity of sugarcane encompasses drought, salinity, temperature, waterlogging and so on (Verma et al. 2020d, e, f; Kumar et al. 2023; Xu et al. 2024). Studies in rice and several other crops revealed that abiotic stresses impart adverse effects on growth and development by influencing the stress-responsive transcription factors, chlorophyll synthesis, photosynthesis,

Table 11.1 List of molecular markers used in sugarcane breeding for crop improvement

Markers	Application of molecular marker	References
AFLP	Analyzing linkage disequilibrium	Raboin et al. (2008)
AFLP	Genetic mapping	Hoarau et al. (2001)
AFLP	Used in genetic assessment for breeding analysis	Lima et al. (2002)
AFLP	Genetic diversity analysis	Asnaghi et al. (2004a, b)
RFLP	Comparison of genetic maps to understand genome structure	Glaszmann et al. (1997)
SNP	Comparison of genetic maps to understand genome structure	Aitken et al. (2014)
SNP	Used to align sugarcane to sorghum	Garsmeur et al. (2018)
SSR	Genetic mapping, genome-wide association mapping, next-generation sequencing	Ming et al. (1998); Aitken et al. (2005, 2007); Thipmongkol et al. (2007); Rossi et al. (2003); Tsuruta et al. (2017); Cardoso-Silva et al. (2014); Zhang et al. (2017)
RAPD	Genetic mapping	Asnaghi et al. (2004a, b)
ILP	Aid in trait association and selection of hybrids	Pongsiripat and Ukoskit (2015)
DArT	Genetic mapping, diversity analysis, genome-wide association mapping	Aitken et al. (2014)
DArT	QTL detection, GWAS application, genetic enhancement	Costet et al. (2012a, b); Wei et al. (2010)
DArT	Analyzing linkage disequilibrium	Wei et al. (2010)
TRAP	QTL mapping for various traits	Liu et al. (2016)
TRAP	Germplasm characterization and diversity analysis	Alwala et al. (2006); Singh et al. (2017)
SCAR	For drought resistance	Srivastava et al. (2012)
Retrotransposon based molecular markers	Genetic mapping	Palhares et al. (2012)
ISSR	For drought resistance	Fahmy et al. (2008)
R-ISSR (RAPD-ISSR)	For drought resistance	Khaled et al. (2017)

(continued)

Table 11.1 (continued)

Markers	Application of molecular marker	References
ISSR	For analyzing genetic polymorphism and similarities	Khaled et al. (2015)
SCAR	For selecting salt-tolerant genotypes	Srivastava et al. (2012)
ISSR	Characterizing the salt-tolerant varieties	Markad et al. (2014)

transpiration, enzyme mechanism, etc. (Singh et al. 2021a, b; Joshi et al. 2022, 2023; Verma et al. 2021a, 2023a, b; Gujjar et al. 2020, 2021, 2024d). Efforts were made to develop varieties that could withstand these stresses (Anas et al. 2021a, b; Krishna et al. 2022; Malviya et al. 2024). The utilization of molecular markers, such as RFLP, ISSR, SSR, RAPD, etc., are widely enhanced for assisting the selection of quantitative trait loci associated with markers in sugarcane, thereby improving crop resilience and productivity (Stuber 1992; Malviya et al. 2021; Verma et al. 2022a, b; Song et al. 2021, 2022).

RAPD and ISSR-PCR techniques have been effectively used to identify molecular markers linked to drought tolerance in sugarcane, with studies showing their association with both salt and drought stress resistance (Fahmy et al. 2008; Singh et al. 2021; Abdel-Tawab et al. 2003a, b; Piperidies et al. 2004). Additionally, the use of SSR markers in breeding programs has significantly reduced the time required for selecting drought-resistant sugarcane varieties by at least 30%, enhancing the efficiency of breeding processes in sugarcane improvement (Hemaprabha and Simon 2012; Prasitsom et al. 2019; Huang et al. 2022; Oliveira et al. 2022). Salinity is another significant abiotic stress that affects the yield of sugarcane by inhibiting the maturation and growth of sugarcane by altering many morphological and physiological mechanisms (de Andrade et al. 2017; Verma et al. 2022c; Singh et al. 2022; Guo et al. 2023). ISSR markers were widely used for characterizing the salt-tolerant sugarcane varieties (Markad et al. 2014; Malviya et al. 2022). RAPD and ISSR markers across various distinct sugarcane varieties demonstrated the possibility of genetic discrimination and marker-assisted selection for finding salt-tolerant varieties and reducing the inefficiencies in breeding programs (Forough et al. 2017; Guo et al. 2022). Along with these markers, sequence-characterized amplification region (SCAR) markers were utilized to select drought-tolerant genotypes in the sugarcane gene (Srivastava et al. 2012).

11.2.2 Biotic Stresses

Biotic stress conditions like herbivory and pathogen attacks in sugarcane can potentially decrease the yield and quality by creating oxidative stress (Manimekalai et al. 2022; Gujjar et al. 2024a). Since sugarcane cultivars are vulnerable to several pathogens, it is difficult for breeding programs to introduce germplasm with high yield, high-sucrose content, robust resilience to biotic and abiotic stressors, and enhanced ratoonability by traditional breeding (Cursi et al. 2021; Verma et al. 2021c, d; Tian et al. 2023; Li et al. 2023). Thus, developing commercial clones with excellent agronomic performance in the context of changing climatic scenarios is the prime thrust area for sugarcane research (Verma et al. 2022d, e). To attain sustainability in the sugarcane production system, the use of novel biotechnological breeding techniques based on numerous molecular markers has to be popularized (Tiwari et al. 2010; Deng et al. 2023; Verma et al. 2024c, d). In sugarcane breeding attempts, it was discovered that SSR, AFLP, and RFLP markers were generally efficient in selecting for resistance to pachymetra Root Rot and Brown Rust (McIntyre et al. 2005). Similarly, for smut, a SCAR marker associated with the smut resistance gene has been identified (Que et al. 2014). AFLP and SSR markers have successfully identified a significant QTL associated with resistance to yellow spot disease in sugarcane (Aljanabi et al. 2007). DArT markers have proven effective in mapping resistance to Sugarcane yellow leaf virus, contributing valuable markers for GWAS in commercial sugarcane varieties and breeding lines (Costet et al. 2012a, b; Wei et al. 2010).

11.3 Emerging Molecular Marker Technologies

11.3.1 Next-Generation Sequencing or High-Throughput Sequencing

The quick and accurate sequencing of millions to billions of DNA or RNA molecules is made possible by high-throughput sequencing (HTS) technology. The introduction of this sequencing method has changed the field of genomics and molecular biology (Ghosh et al. 2018). HTS enables comprehensive analysis of genetic material and thus offers previously unattainable insights into the organism's genetic composition (Graveley 2008). HTS technology has many applications, including whole genome sequencing, identifying specific genes, analyzing transcriptome profiles, etc., over the traditional sequencing method (Raju et al. 2020). Another advantage of this technology is the ability to detect different pathogens and novel pathogenic agents in sugarcane and thus enhance disease management (Van Dijk et al. 2014; Loman and Pallen 2015; Singh et al. 2023; Verma et al. 2024e). Additionally, HTS speeds up the breeding of disease-resistant cultivars by facilitating a better knowledge of plant-pathogen interactions and assisting in the

identification of genetic markers for disease resistance (Shafi et al. 2019; Solanki et al. 2023). HTS has been utilized for the identification of novel genes and transcripts that are critical for stress tolerance. Breeders may use the ability to detect and investigate thousands of upregulated transcripts at high temperatures, including ferredoxin-dependent glutamate synthase, stress protein DDR-48, and phytepsin, to discover genetic targets that will help develop sugarcane types that can withstand high temperatures. This knowledge may be utilized to maintain sustainable sugar production (Verma et al. 2022f, g), strengthen crop resilience, and improve breeding techniques under stress conditions (Raju et al. 2020; Zheng et al. 2023; Bhatt et al. 2023a, b) [Table 11.1].

Advances in next-generation sequencing (NGS) technologies have significantly reduced the cost per unit data in sequencing studies, particularly in single nucleotide polymorphism (SNP) detection, enabling the use of a very large number of markers in studies involving association mapping in sugarcane (Hirschhorn and Daly 2005; Syvanen 2005; Bhatt et al. 2024). Hundreds of thousands of SNPs may now be identified in genome-wide association studies (GWAS) of complex characteristics by sequencing the entire genome of a chosen panel of genotypes that differ substantially (Banerjee et al. 2020). NGS has evolved into a potential technique for gene identification and transcriptome profiling analysis (Raju et al. 2020; Chen et al. 2023). The omics approaches, such as transcriptomics, help in elucidating the interconnections among the complex genetic makeup, proteins, genes and metabolites of sugarcane and aid in crop improvement and better energy cane development (Mustafa et al. 2018; Verma et al. 2023c, d).

11.3.2 Omics Approaches

Omics approaches provide an idea of the mechanism adopted by crops due to various stresses and help in selecting QTLs that provide tolerance to respective stresses (Kumar et al. 2023). Through a variety of in silico approaches, such as probe hybridization array, expressed sequence tags (ESTs), or known genes of other related crops, transcriptome analysis offers the necessary information regarding genes (Srivastava 2020). Transcriptomic studies in sugarcane have identified important genes and mechanisms involved in the plant's response to various pathogens, such as *Sporisorium scitamineum*, *Puccinia melanocephala*, and *Fusarium verticillioides*. Various studies revealed that differentially expressed genes (DEGs) are associated with crucial biological processes, including hormone signaling pathways, phenylpropanoid biosynthesis, and nitrogen metabolism, which play a significant role in disease resistance and stress response in sugarcane (Huang et al. 2018; Avellaneda et al. 2018; Lin et al. 2016; Wang et al. 2019; Verma et al. 2021e, f). Transcriptomic studies also showed vital genes and transcription factors associated with drought tolerance, like ascorbate peroxidase, MYB, and aquaporin, which were upregulated in drought-tolerant cultivars (Belesini et al. 2017) (Fig. 11.1).

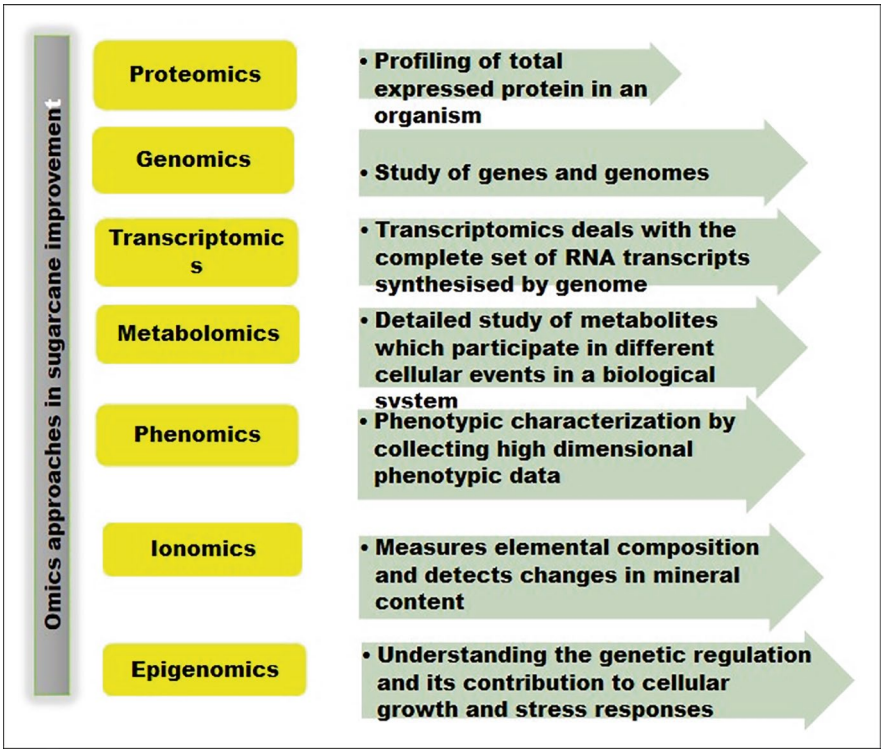

Fig. 11.1 Different omics approaches employed in sugarcane crop improvement strategies

Plant biology research now heavily relies on metabolomics, the study that examines how phenotypic and physiological changes respond to external influences (Sumner et al. 2003). Since it may identify similarities between parent and offspring lines, the application of metabolomics in sugarcane can be crucial (Ferreira et al. 2018). Understanding the relationship between sugar-related genes and the proteins involved in sugar synthesis is crucial in metabolomics research (Mahlanza et al. 2019). Metabolomics approaches can also be utilized to decipher the plant–pathogen interaction and resistance mechanism (Correr et al. 2019; Carreno-Quintero et al. 2013). Along with transcriptomics and metabolomics, proteomics also has significance in sugarcane crop improvement since it allows for the detailed analysis of a plant's protein expression, which helps in the identification of key proteins involved in the stress responses, growth, and development (Barnabas et al. 2015; Singh and Shami 2018; Gujjar et al. 2024a, b, c). Similarly, the ionomics approach is used to understand the trace elements and essential mineral nutrient availability to plants and aid nutrient profiling (Kumar et al. 2023). It is essential to comprehend how whole-plant management of nutrient intake, distribution, and stress responses occurs in plants such as sugarcane. Integrated analysis of phenome, ionome, transcriptome, proteome and metabolome will aid in elucidating the physiological and

molecular mechanisms related to many challenges in sugarcane (Gujjar et al. 2024b, c).

11.4 Molecular Markers Associated with Various Traits in Sugarcane

Molecular markers are potential tools in modern sugarcane breeding, mainly for identifying and predicting specific phenotypic traits. Accurately tagging and validating the marker or markers closely connected to the trait(s) of interest is necessary for using molecular markers to predict phenotype. Trait-associated stable markers, or even mapped markers, may economically benefit marker-assisted breeding programs (Siraree et al. 2017). Traditionally linkage mapping was utilized for tagging segregating traits in crops. Although effective linkage mapping has produced many examples, quantitative trait loci (QTLs) in sugarcane have been found sparingly during the past few decades. This is due to the complex genome of sugarcane (Siraree et al. 2017). Linkage disequilibrium (LD)-based mapping, or association mapping (AM), has gained popularity among researchers as a solution to the problems with traditional linkage mapping (Table 11.1).

This method identifies QTLs by analyzing the degree of marker-trait associations that have remained stable despite the genetic reshuffling that occurs during evolutionary recombination within a species (Banerjee et al. 2015; Singh et al. 2016). Various molecular markers have been crucial in identifying and associating various traits in sugarcane, which helps in precise breeding. For instance, markers connected to various diseases resulting from biotic stress in sugarcane have been utilized to develop sugarcane varieties resistant to particular stress. The R12H16 and 9O20-F4 are two markers that are closely interconnected for resistance to the brown rust Bru1 gene, and similarly, the G1 marker is closely linked to orange rust diseases in sugarcane (Le Cunff et al. 2008; Yang et al. 2018). Single nucleotide polymorphism (SNP) microarrays have been utilized to localize target traits and identify QTLs related to various biotic stress resistance and agronomical traits in sugarcane, such as yellow leaf, ratoon stunting, leaf blight, mosaic disease, chlorophyll content, tillering, and ratooning ability, though none have yet progressed to practical application (You et al. 2019, 2021; Wang et al. 2022, 2023a).

11.5 Integration of Molecular Markers with Genomic Selection

Genomic selection (GS), also known as genome-wide association study (GWAS), is a novel strategy used to identify genetic variations that influence complex traits through genome-wide comparative analysis (Tibbs Cortes et al. 2021). The

traditional method usually finds statistically significant correlations between genotype and phenotype to discover certain genomic areas that regulate the variance of quantitative characteristics (Lu et al. 2024). Moreover, molecular markers consolidated with GS coordinating all accessible markers to make genomic prediction models that may replace the chore of conventional phenotypic determination in breeding programs more sophistically (Sandhu et al. 2022; Islam et al. 2023). Generally, GS is used to investigate traits including yield, sugar content, fiber composition, drought tolerance, and disease resistance in sugarcane (Zhang et al. 2023a, b; Wirojsirasak et al. 2023).

11.5.1 Breeding Strategies

Molecular breeding strategies in sugarcane have evolved significantly with advancements in genetic tools and technologies, aiming to overcome the challenges posed by the crop's complex polyploid genome. These strategies, which include marker-assisted selection (MAS), marker-assisted backcrossing (MABC), and bulked segregant analysis (BSA), are increasingly integrated with high-throughput genotyping, genomic selection (GS), and machine learning to enhance breeding efficiency and precision.

11.5.2 Marker-Assisted Selection (MAS)

MAS has become a critical tool in modern sugarcane breeding, particularly in what is often referred to as the "Breeding 3.0" era. This approach uses molecular markers closely linked to traits of interest, such as disease resistance or high-sugar content, to guide the selection of desirable genotypes offering high yields (Sharma et al. 2024a, b). MAS provides a reliable and efficient breeding method, largely unaffected by environmental factors (Lu et al. 2024). MAS has increased sugar yield and cane production in sugarcane while minimizing disease outbreaks. Increasing sugar content and cane production while reducing the risk of disease outbreaks can be achieved at little expense by selecting and planting disease-resistant genotypes (Aitken 2022). However, the complexity of the sugarcane genome has limited the success of MAS, mainly due to the difficulty in capturing all the genetic variations responsible for complex traits. Despite these challenges, the future of MAS in sugarcane breeding lies in its integration with high-throughput genotyping, GS, and machine learning, which promise to enhance the accuracy and productivity of breeding programs (Sandhu et al. 2022).

11.5.3 Marker-Assisted Backcrossing (MABC)

To address some of the limitations of MAS, sugarcane breeders have also turned to marker-assisted backcrossing (MABC). This novel approach elevates the traditional backcrossing method by implementing potential molecular markers to accelerate the recovery of the recurrent parent phenotype, thereby depleting the number of backcrosses needed. This approach is particularly useful in transferring specific traits from one variety to another, such as disease resistance, while retaining the overall characteristics of the elite variety (Tanksley et al. 1989). MABC, along with marker-assisted recurrent selection (MARS), continues to be a valuable tool in sugarcane breeding, helping to introduce beneficial alleles into commercial varieties more efficiently.

11.5.4 Bulked Segregant Analysis (BSA)

Bulked segregant analysis (BSA) is another molecular breeding strategy used in sugarcane to identify markers associated with specific traits (Giovannoni et al. 1991; Michelmore et al. 1991). BSA involves creating two pools of individuals with extreme phenotypes from a segregating population and then comparing the allele frequencies between these pools to identify significant differences (Wang et al. 2023b). This method is cost-effective and simplifies the process of locating markers linked to target traits. In sugarcane, BSA has been used to identify markers for traits such as resistance to brown rust, smut, leaf blight, and brown stripe (Wang et al. 2023b; Asnaghi et al. 2004a, b; Xu and Chen 2004; Gao et al. 2022; Cheng et al. 2022). However, the application of BSA in sugarcane is still limited due to the complexity of its genome, making the identification of markers for many traits challenging (Lu et al. 2023).

11.6 Genome Editing

Genome editing (GE) alters the genome via insertion, deletion or replacement of DNA by using designed nucleases that create site-specific double-strand breaks at the desired locations to make specific alterations (Mohan 2016). Mega nucleases, zinc finger nucleases (ZFNs), transcription activator-like effector nuclease (TALEN), and clustered regularly interspaced short palindromic repeats (CRISPR/Cas9) are the four crucial engineered nucleases widely utilized in the genome editing approaches (Basak et al. 2021; Kumar et al. 2023). The CRISPR/Cas system has outperformed other GE technologies, including ZFNs and TALENs, due to its ease of use, affordability, and efficacy (Mohan 2016).

11.7 CRISPR/Cas System

The CRISPR/Cas9 method has been widely used in sugarcane breeding to develop novel varieties possessing desirable traits (Amaresh et al. 2024). This method is utilized to fuse several genes, resulting in effective metabolic engineering and biological improvement that will guarantee long-term improvement in sugarcane yield. This simple approach of RNA-guided genome editing has developed into a cutting-edge technology with a wide range of biological applications, including the potential to successfully induce specific genomic alterations in plant tissues (Hussin et al. 2022). The primary step in the genome editing method by CRISPR/Cas9 technology involves the target identification and designing of single guide RNA complementary to the target DNA sequence (Li and Iqbal 2024). The gRNA contains two regions: the recognition lobe (REC) and the nuclease lobe (NUC). This REC lob has two multi-helix domains, which are important in the binding of designed gRNA and target DNA, which are known as REC1 and REC2 (Xing et al. 2014; Ren et al. 2021). During the target recognition, gRNA binds to the Cas9 protein and serves as a molecular scissor (Xing et al. 2014). RuvC, HNH, and PAM (protospacer adjacent motif) are three interacting domains in the NUC lobe that help in the target binding and precise cleaving (Li and Iqbal 2024) [Fig. 11.2 and 11.3].

REC lobe helps bind gRNA and target binding, while RuvC and HNH domains facilitate the precise cutting of target DNA's complementary and non-complementary strands (Li and Iqbal 2024). These components of the CRISPR genetic system have to be transferred into the plant's genome by various delivery techniques, including agrobacterium-mediated infection, agro-infiltration, particle bombardment, electroporation, virus-mediated transformation, PEG-based transformation, etc. (Zaidi et al. 2017; Bhowmik et al. 2018). Cas9 protein, with the aid of gRNA, causes DNA cleavage, creating a break in the DNA at that exact site (Svitashev et al. 2015; Galli

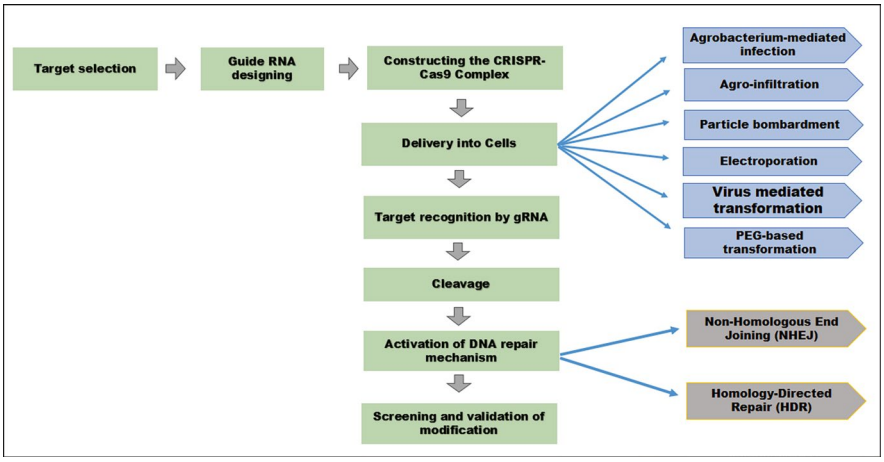

Fig. 11.2 Steps involved in CRISPR/Cas9 mediated genome editing method

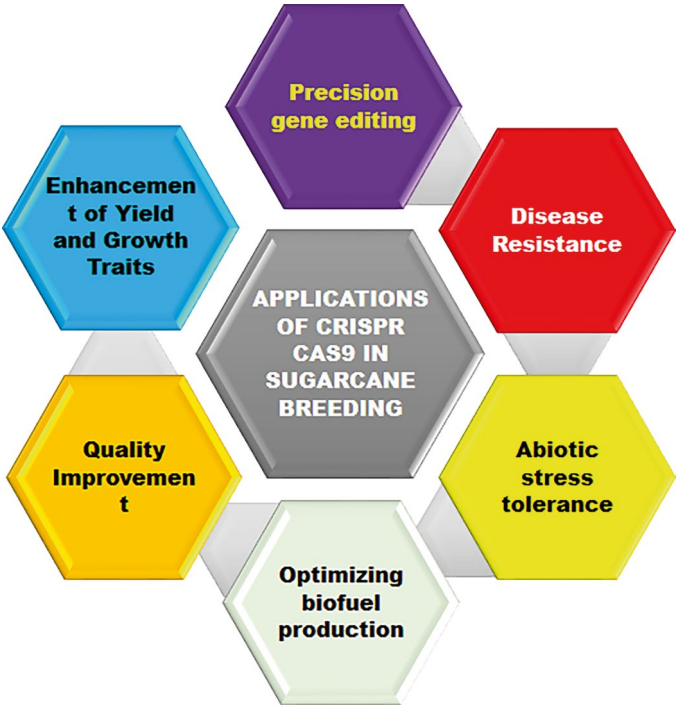

Fig. 11.3 Applications of CRISPR/Cas9 technique in sugarcane breeding

et al. 2022). The DNA cleavage frequently triggers the cell's natural repair processes, which try to repair the break using non-homologous end joining (NHEJ) or homology-directed repair (HDR) (Li and Iqbal 2024). NHEJ acts by gene disruption followed by additions or deletions, whereas HDR works by initiating specific genetic modifications (Ren et al. 2021; Xing et al. 2014; Tang et al. 2017; Miao et al. 2018).

CRISPR/Cas9 technology has evolved as an effective tool in sugarcane genome editing, as it can easily modify several potent genes for many significant agronomic traits (Kannan et al. 2018). This technique has been a useful editing tool that can be employed in polyploidy crops like sugarcane with the aim of enhanced sucrose accumulation, biofuel production, biotic and abiotic stress tolerance, enhancement of yield traits such as leaf parameters, tiller number, photosynthetic rate etc. (Azevedo et al. 2011; Taparia et al. 2012; Hoang et al. 2015; Ali et al. 2019; Amaresh et al. 2024). Precision gene modification is the most popular specific use of CRISPR/Cas9 in sugarcane, which involves targeting a particular DNA sequence with gRNA and cutting the DNA with the Cas9 enzyme (Sharma et al. 2024a, b).

11.8 Future Perspective

Developing and implementing molecular markers in sugarcane breeding was a pivotal moment. The shortcomings of conventional breeding techniques can be overcome up to an extent by using molecular marker techniques. Although there are challenges like high cost and high resource requirements for developing and validating markers according to the requirements, including ethical and regulatory concerns, molecular markers will continue to evolve. More recent studies revealed the significance of integrating molecular markers with various omics technologies like proteomics, metabolomics, etc., to create abiotic and biotic stress-tolerant and resistant varieties. Similarly, by integrating molecular markers and machine learning, artificial intelligence is expected to have great potential and revolutionize breeding efforts to improve the desired agronomic traits. With the advancement of technologies, the incorporation of cutting-edge approaches in sugarcane breeding will accelerate the improvement and development of novel varieties.

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Chapter 12
Use and Misuse of Markers in Sugarcane Breeding

Khushboo Jain, Ramswaroop Saini, Ayushi Malik, Avinash Marwal, Mayank Suthar, and Krishnaveer Singh Jhala

Abstract Markers are individual nucleotides that indicate a particular gene; the importance of selection, trait mapping, and region mapping is known. Sugarcane is a complex hybrid interspecific cross with polyploidy in nature. Five to 16 different species of the *Saccharum* genus have been used for sugarcane breeding, due to which sugarcane has a complex genome structure and genetic background. Genome analysis is important for genomic selection, and analyzing single nucleotide polymorphism (SNP) markers in breeding chromosome segment substitution lines (CSSLs) is important for selecting desirable traits. Marker-assisted selection is an efficient tool for selection as it focuses on screening quantitative trait loci (QTLs) and mapping the traits affecting the scenario. Genotyping of markers is done for QTL mapping, which identifies markers, such as SSRs, SNPs, etc. After genotyping and identification of markers, breeding candidates are selected, and further phenotyping is done for favorable genotypes. Favorable genotypes are tested for progeny vigor and are further analyzed for year-wise sugar recovery. The beneficial sugarcane traits are introduced in a new elite variety through molecular characterization techniques such as SSR and SNP-based markers since they are easy to design and more efficient. Generating new cultivars involves the application of biotechnology, which consists of the induction of mutations, which is done by chemical mutagens and depends on stress mechanisms. This review focuses on using and misusing various markers, their applications, challenges, and ethical considerations in sugarcane breeding programs.

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Keywords Molecular markers · Crop improvement · Disease resistance · Stress tolerance · Increased yield

12.1 Introduction

Sugarcane (*Saccharum* spp. hybrids) is a major global crop valued economically as a sugar and bioenergy source. China ranks third in the world in sugar production. Sugarcane is a perennial grass known for its high photosynthetic efficiency rapid biomass accumulation, and complex genetic makeup, which includes aneuploid polyploidy (≥8 chromosomes) and genetic heterogeneity. Conventional breeding methods, primarily involving stem cutting, are labor-intensive and time-consuming, often taking decades to develop new varieties. The genetic complexity of sugarcane cultivars arises from the interspecific hybridization between *S. spontaneum* and *S. officinarum* (Garsmeur et al. 2018), where commercial cultivars inherit ~70–80% of their chromosomes from *S. officinarum*, ~10–15% from *S. spontaneum*, and ~5–10% from interspecific recombination.

This limited genetic base may explain the slow progress and improvement in sugarcane breeding. Despite sugarcane's economic significance, genetic research in this crop has been relatively underdeveloped due to challenges such as high heterozygosity, longer breeding cycles, and the difficulties associated with traditional plant breeding methods. For successful breeding, careful characterization and germplasm assessment, along with a strong knowledge of breeding techniques, are essential (Aitken 2022). Sugarcane, an outbreeding crop, is susceptible to inbreeding depression. Its highly heterozygous genome, with amplified polymorphisms due to high ploidy, complicates breeding efforts. Early hybridizations of *S. spontaneum* clones, which have a cytotype of $x = 8$ (Arceneaux 1965), have led to a narrow genetic base. Most commercial varieties today trace their lineage to a small number of founding ancestors, resulting in limited recombination opportunities during meiosis. This has led to extensive linkage disequilibrium (LD) in breeding populations, contrasting with the genetic diversity observed in other outbreeding crops like maize. In addition, sucrose content remains the primary commercial trait for sugarcane improvement. Identifying genetic markers associated with sugar yield is a critical strategy in breeding programs. The mapping of genes controlling yield components, such as sucrose content, cane yield, stalk diameter, stalk number, and resistance to diseases and environmental stresses has shown promise in enhancing sugarcane productivity (Margarido et al. 2015; Balsalobre et al. 2016; Balsalobre et al. 2017). To address these challenges, modern biotechnological techniques, including molecular marker-assisted breeding, genetic transformation, genome editing, and multi-omics approaches, have been developed to enhance sugarcane yield, sugar content, disease resistance, and stress tolerance (Sahu et al. 2014; Mustafa et al. 2018; kumar et al. 2024).

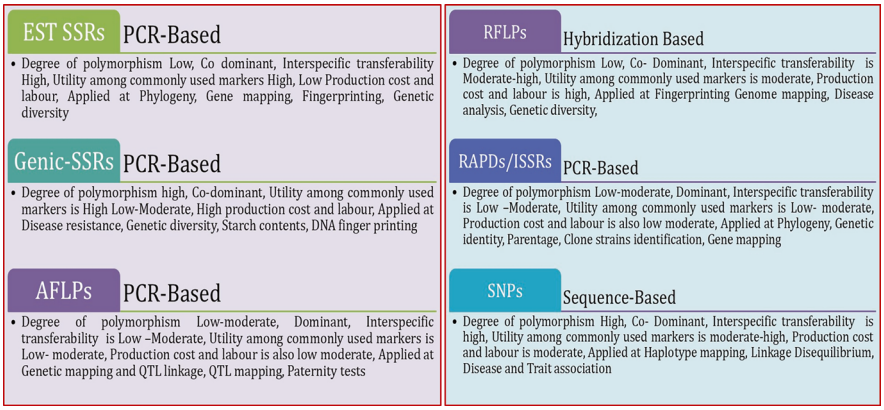

Fig. 12.1 Comparative presentation of different molecular markers used in sugarcane breeding development

Among these, molecular marker technologies have played a pivotal role in developing cultivars with desirable traits, such as high yield, disease resistance, herbicide resistance, and tolerance to biotic and abiotic stresses. (Maheshwari et al. 2023; Mishra et al. 2024). These technologies have been employed for genetic diversity studies, molecular-assisted selection (MAS), paternity analysis, quantitative trait loci (QTL) mapping, cultivar identification, phylogenetic studies, and genetic mapping. Several types of molecular markers, including simple sequence repeats (SSRs), inter-simple sequence repeats (ISSRs), restriction fragment length polymorphisms (RFLPs), random amplification of polymorphic DNAs (RAPDs), amplified fragment length polymorphisms (AFLPs), and single nucleotide polymorphisms (SNPs), are actively being used in sugarcane genetic research (Fig. 12.1). Marker development in sugarcane has evolved through three distinct phases (Marwal and Gaur 2020a; Aitken 2022).

12.1.1 Early Phase of Marker Development

The early phase of marker development in sugarcane involved using complex and labor-intensive methods to visualize and generate small numbers of markers, often requiring high-level technical expertise for consistent results. The primary techniques used were restriction fragment length polymorphism (RFLP) and cDNA RFLP.

12.1.1.1 Advantages of Early Phase Marker Development Techniques

- Single-dose markers facilitated genetic mapping in polyploid sugarcane.
- Cross-species comparisons revealed structural similarities between sugarcane and other grasses.
- RFLP markers provided valuable insights into chromosome pairing during meiosis.
- These markers helped assess genetic diversity in sugarcane clones, supporting further genetic research.

Disadvantages of Early Phase Marker Development Techniques
- RFLP-based mapping was slow and limited in terms of the number of clones that could be studied.
- Early maps offered uneven and low genome coverage.
- Sugarcane's high heterozygosity and inbreeding depression made generating inbred populations difficult.
- Early marker data primarily contributed to genetic research rather than immediate breeding improvements.

12.1.1.2 PCR-Based Marker Development

The next phase saw the introduction of PCR-based markers, which significantly improved the ease of marker generation and allowed the production of hundreds of markers. Despite their advantages, these markers, such as random amplified polymorphic DNA (RAPD) and amplified fragment length polymorphism (AFLP), still required manual scoring and exhibited some limitations. RAPD markers, for example, were fast to generate but lacked repeatability and had low transferability across different populations or laboratories.

Advantages of PCR-Based Markers
- PCR-based markers are robust, reproducible across labs, and suitable for large-scale screening of sugarcane populations.
- They generate large numbers of single-dose markers, improving genetic map coverage and genetic diversity analysis.
- These markers enabled studies of large populations (e.g., 430 *S. spontaneum*), providing insights into genetic relationships and clustering patterns.
- Diversity array technology (DArT) provided high-throughput, array-based approaches for marker generation and was cost-effective for QTL and GWAS studies.
- SSR markers were instrumental in clonal verification, ensuring propagation accuracy and intellectual property protection.

Disadvantages of PCR-Based Markers
- AFLP markers required skilled handling and were difficult to align across genetic maps.

- DArT markers often showed low polymorphism in crosses between commercial varieties, limiting their utility.
- Transferring AFLP markers to capillary sequencing platforms presented challenges due to reduced fragment sizes, which affected marker detection and scoring accuracy.

12.1.1.3 DNA Sequence-Based Markers

The current phase of marker development in sugarcane has shifted toward using high-throughput sequencing technologies, initially developed for diploid crops, to identify polymorphic SNPs and insertion–deletions (indels) across the sugarcane genome. This approach capitalizes on the abundance of genetic variation present in sugarcane, enabling more efficient genetic mapping.

Advantages of DNA Sequence-Based Markers
- High-throughput sequencing facilitates the identification of thousands of SNPs, enhancing genetic mapping.
- The combination of DArT, SNP, and SSR markers significantly increases genetic map resolution (92% linkage group assignment).
- Genotype-by-sequencing eliminates the need for prior sequence knowledge and is scalable for large populations.
- Identifying SNPs across diverse sugarcane accessions aids in understanding genetic diversity.

Disadvantages of DNA Sequence-Based Markers
- Techniques such as Sequenom assays and 454 sequencing are expensive.
- Despite identifying large numbers of SNPs, the cost of applying them in breeding programs remains high.
- Genotype-by-sequencing requires advanced bioinformatics expertise to process and interpret the data.
- Developing SNP arrays requires substantial sequence data, making the process costly and complex.
- Validating large numbers of SNP markers for practical breeding applications is resource-intensive.

12.2 Types of Genetic Markers Used in Sugarcane Breeding

Molecular markers have become indispensable tools in sugarcane breeding and genetic improvement, facilitating more efficient germplasm characterization, trait mapping, and marker-assisted selection (MAS). These markers are particularly valuable for genetic diversity assessment, parentage analysis, quantitative trait loci (QTL) mapping, cultivar identification, phylogenetic analysis, and genetic mapping. Among the various molecular markers employed in sugarcane research are

simple sequence repeats (SSRs), inter simple sequence repeats (ISSRs), restriction fragment length polymorphisms (RFLPs), random amplification of polymorphic DNAs (RAPDs), amplified fragment length polymorphisms (AFLPs), and single nucleotide polymorphisms (SNPs). Each of these marker types has unique advantages and applications that contribute to the advancement of sugarcane breeding and genetics (Ahmad et al. 2018; Pan 2010).

12.2.1 Simple Sequence Repeats (SSRs)

Simple sequence repeats (SSRs), another name for microsatellite sequences, are made up of 1–6 base pair motifs repeated 5–50 times and surrounded by distinct but conserved genome sequences (Pan 2017). SSRs are classified according to the type of repetition and the quantity of repeated base pairs. They exhibit many kinds of repetition patterns and can be mono-, di-, tri-, tetra-, penta-, or hexa-SSRs, as well as perfect, imperfect, or compound SSRs. Depending on where they are located in the genome, SSR markers can be categorized as nuclear (nuSSR), mitochondrial (mtSSR), or chloroplast (cpSSR) SSRs. By demonstrating transcript isoforms and facilitating the identification of novel genes, ESTs (expressed sequence tags) have greatly improved gene identification in sugarcane. The SUCEST database (sugarcane ESTs) has been used to identify over 5000 new SSRs.

Conventional techniques for SSR isolation, such as creating and screening genomic libraries, are expensive and time-consuming. Alternative methods have been developed to speed up SSR separation and expand the number of SSR loci, such as next-generation sequencing, EST libraries search, and enriched-genomic libraries search. Polymorphic SSRs are frequently identified using fragment analysis by capillary electrophoresis and SSR-capillary electrophoresis/fluorescence detection (SSR-CE/FD). These techniques make accurate scoring of highly variable SSR loci possible, providing an accurate and efficient SSR marker test (Pan 2010, 2017. SSR-CE/FD, which uses capillary electrophoresis and fluorescence detection of SSR fragments, is more complicated, time-consuming, and costly than polyacrylamide gel electrophoresis with silver staining.

12.2.2 Single Nucleotide Polymorphisms (SNPs)

Single-nucleotide polymorphisms (SNPs) are of enormous functional importance since they either fully associate with particular features or contribute to phenotypic polymorphisms. SNPs often occur in plant genomes at a frequency between one per 100 and 500 base pairs, with species-specific variations found. When a nucleotide in a particular accession differs from the reference genome at the same nucleotide position, it can be recognized as an SNP. Variants can be found without a reference genome by using de novo assembly techniques to compare the reads from different genotypes. Due to their high genome density, ability to detect allelic dosage (Afzal

et al. 2018), low error rates, and decreased expenses and time-related to their detection (Bundock et al. 2009), SNPs have become the preferred markers for crops. Additionally, they unambiguously make it easier to determine the degree of polyploidy and allelic dosage in complex genomes.

When using short reads, the likelihood of incorrect SNP identification increases; alignment is frequently confused by very similar sequences, leading to homologous polymorphisms being mistakenly classified as real SNPs (Korani et al. 2019). On the other hand, lengthy reads extracted from individual DNA molecules have greatly enhanced the detection of structural variants and the discovery of precise SNPs. (Li et al. 2020). Paired-end reads exceeding 150 base pairs have been shown to effectively differentiate homologous SNPs from allelic SNPs in allopolyploid species, as demonstrated by Clevenger et al. (2015), and are essential for the identification of high-quality SNPs (Zhao et al. 2019). Recently, a haplotype-based approach has been introduced for precise SNP discovery in the subgenome-specific sequences of polyploid organisms.

This strategy utilizes long-read technology, enabling high-resolution haplotypes, and improving mapping accuracy by targeting haplotypes of various lengths and large insert sizes (Clevenger et al. 2018). The application of high-throughput RNA sequencing (RNA-seq) for transcriptome analysis enables the identification of many SNP markers, which support the mapping of desirable traits and enhance marker-assisted selection, especially when integrated with the established sugarcane reference genome. In a study on sugarcane, transcriptome sequencing of six genotypes from biparental crosses identified 708,125 SNPs, which have been proposed as crucial resources for marker identification and genome assembly (Cardoso-Silva et al. 2014).

Furthermore, the transcriptome data of sugarcane have been used as a reference for the assembly of genotyping by sequencing (GBS) data, revealing approximately 442,152 putative gene-based SNPs. These SNPs were identified from various sugarcane varieties under stress conditions such as drought, leaf abscission, and infection by Pokkah boeng disease (Balsalobre et al. 2017). Additionally, over 87,000 SNPs were detected from differentially expressed transcripts in Illumina reads obtained from the sugarcane variety Co 86032, which was exposed to oxidative stress (Gayathri et al. 2019). These findings underscore the significant role of transcriptome sequencing in providing large-scale SNP markers that contribute to identifying traits and advancing breeding programs.

12.2.3 Insertion–Deletion Markers (InDel)

In a study investigating the population structure of 97 elite and historic sugarcane varieties, 6534 insertion–deletion (InDel) and single nucleotide polymorphism (SNP) markers revealed a pronounced structural differentiation, with the varieties clustering into two primary subgroups. A significant correlation was also found between these markers and sucrose-related traits, underscoring their potential value

in breeding programs focused on improving sugar content. As a photosensitive crop, Sugarcane requires specific environmental conditions for optimal growth and development, which can vary significantly across agro-climatic zones, particularly in tropical and subtropical regions. The spatial variation in sugarcane growth is well-documented, and studies focusing on association mapping at a single location have often identified markers that are not universally applicable across diverse environments, limiting their utility in breeding programs.

In a separate investigation, Srivong et al. (2019) characterized 17 sugarcane genotypes from Hawaii and Thailand using 12 target region amplification polymorphism (TRAP) markers combined with partial Sai nucleotide polymorphism analysis. The study generated a total of 275 fragments, of which 273 (99.27%) were polymorphic. The polymorphic information content (PIC) values ranged from 0.912 to 0.959, with an average of 0.938, indicating a high level of genetic diversity among the genotypes. The genetic similarity (GS) values, calculated using Dice's similarity coefficient, ranged from 0.19 to 0.81, with a mean of 0.44. A similar analysis was performed on 14 Thai sugarcane genotypes, each exhibiting different sugar content, revealing a total of 198 fragments, with 174 (87.88%) classified as polymorphic. The PIC values for these genotypes ranged from 0.860 to 0.937, with an average of 0.914. The GS values for these genotypes ranged from 0.49 to 0.85, with an average of 0.71. Dendrograms constructed using the unweighted pair group method with arithmetic mean (UPGMA) clustering indicated clear differentiation between the Thai and Hawaiian sugarcane genotypes, with *Saccharum officinarum* showing the closest genetic relationship to modern sugarcane varieties.

Further analysis of the partial Sai nucleotide sequences from 16 sugarcane genotypes, which spanned 608 to 692 base pairs in exon 2, revealed 49 single nucleotide polymorphisms (SNPs) and two insertion/deletion (In/Del) events. These sequences were used to generate phylogenetic trees, which provided a more refined understanding of the genetic relationships among the genotypes. Interestingly, the results from the nucleotide sequencing exhibited a high degree of similarity to those obtained from TRAP markers. The integration of TRAP and Sai nucleotide sequencing methodologies enhances the ability to identify genetic variations and improve the selection of diverse parent varieties with desirable sucrose content traits for breeding programs. This approach holds significant potential for advancing sugarcane breeding by enabling more targeted and informed selection based on genetic markers associated with sucrose yield.

12.3 Applications of Markers in Sugarcane Breeding

A molecular marker is a specific locus in an organism's genome where nucleotide sequences vary between individuals within a population. Compared to traditional phenotypic and biochemical markers, which were initially used in sugarcane improvement, molecular markers offer several advantages. These include their ease of use, widespread distribution across the genome, and the ability to conduct rapid

screenings (Winter and Kahl 1995). In sugarcane breeding, molecular markers are precious for genetic diversity analysis, genetic linkage map construction, mapping quantitative trait loci (QTLs), map-based gene cloning, and marker-assisted selection (MAS). Numerous studies have utilized molecular markers such as RAPDs, AFLPs, RFLPs, SSRs, ISSRs, SNPs, and TRAPs to assess genetic variation and genetic relationships within sugarcane populations (Fig. 12.2) (Ali et al. 2019; Manimekalai et al. 2020). For example, genetic diversity studies of *Saccharum* genus accessions, based on phenotypic traits and pedigree analysis, have demonstrated the effectiveness of microsatellite (SSR) markers and in a survey of 66 sugarcane accessions, six SSR markers revealed 187 distinct alleles, highlighting the utility of SSR markers for investigating genetic diversity in modern sugarcane cultivars, particularly those derived from inter-specific hybridization (Cordeiro et al. 2003).

In another study, examining the genetic relationships among 82 sugarcane varieties unaffected by environmental factors, AFLP, TRAP, and SSR markers were employed. A total of 410 polymorphic markers were identified across the three marker types, with AFLP and TRAP markers showing higher genetic similarity (0.675 and 0.655, respectively) compared to SSRs (0.522). Moreover, the coefficient of variation was lower for AFLP and TRAP markers (~6.55%) than for SSR markers (13%), indicating that the choice of a molecular marker is critical and that no single marker system is universally suitable for germplasm characterization in breeding programs (Creste et al. 2010). Further genetic diversity analysis conducted by Nayak et al. (2014) on 1002 sugarcane accessions and related grasses, using 36 SSR markers, identified 209 alleles associated with important agronomic traits, including biomass and stress tolerance, further reinforcing the importance of SSR markers in genetic studies of sugarcane. A recent survey of sugarcane breeding in China analyzed the genetic diversity of 150 popular sugarcane accessions using 21 fluorescence-labelled SSR markers and high-performance capillary electrophoresis (HPCE). The results of this study demonstrated the importance of combining SSR

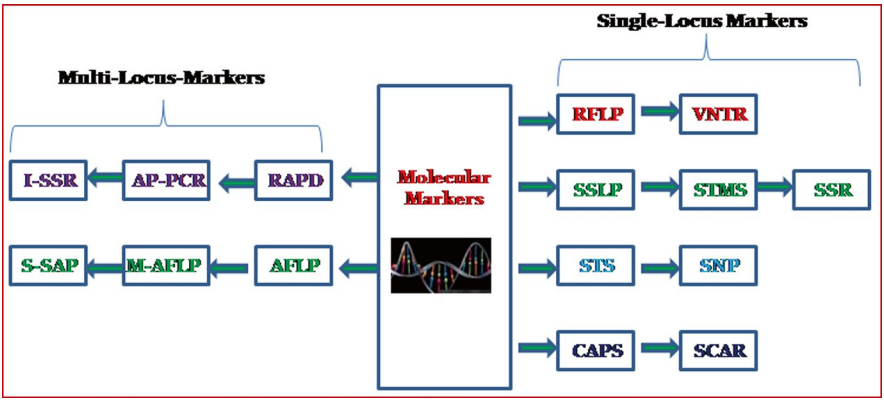

Fig. 12.2 Different types of molecular markers used in sugarcane breeding

markers with HPCE, particularly for the genotyping of polyploid sugarcane. This combination provides valuable insights for sugarcane breeders, assisting in managing parental germplasm, selecting optimal parents for crossing, and producing progeny with enhanced traits, such as higher yield, disease resistance, and tolerance.

Molecular markers, also known as DNA markers, have become crucial tools for studying the genome structure of sugarcane, facilitating the identification of quantitative trait loci (QTLs) and the construction of genetic maps based on recombination frequencies between markers in the genome (Pinto et al. 2010). Early studies focused on developing incomplete genetic or linkage maps using F1 progeny from sugarcane varieties such as *S. officinarum*, *S. spontaneum*, and some modern cultivars. For instance, using 72 SSR and 40 AFLP markers in such progeny identified 28 repulsion linkages (Zhou et al. 2021). Another study aimed at constructing a genetic linkage map for *S. officinarum* and *S. spontaneum* accessions using AFLP, SRAP, and TRAP markers concluded that AFLP markers were particularly effective due to their genome-wide distribution. However, SRAP and TRAP markers, by targeting gene-rich regions, were helpful for QTL mapping (Alwala et al. 2008). Whole genome mapping of 183 sugarcane accessions, covering important traits such as morphology, disease resistance, bagasse residual content, and cane yield, used DArT (Diversity Arrays Technology), SSR, and AFLP markers. Despite its polyploidy, this study demonstrated the feasibility of using genome-wide association studies (GWAS) in sugarcane, emphasizing the importance of considering family relatedness alongside population structure.

12.3.1 Applications of Markers in Improving Disease Resistance

The genetic complexity of sugarcane, including multiple alleles for single genes, presents challenges for genetic and linkage mapping using molecular markers. Despite these complexities, QTL mapping with RFLP, AFLP, and SSR markers has successfully identified several loci associated with disease resistance (Aitken 2016. Some key diseases affecting sugarcane include smut disease, Fiji leaf gall, leaf scald disease, pachymetra root rot, brown rust, and yellow leaf disease. Yellow leaf disease, caused by the sugarcane yellow leaf virus (SCYLV), has become a global concern, leading to significant yield losses. Several studies have aimed to identify the genetic factors responsible for resistance to SCYLV (Marwal and Gaur 2020b). In one such study, 196 progenies from a cross between the susceptible cultivar R570 and the resistant cultivar MQ76–53 were genotyped with AFLP and SSR markers, identifying 2822 polymorphic markers. The results indicated that the SCYLV resistance in MQ76–53 was controlled by a significant quantitative trait allele (Ryl1), which accounted for 32% of the phenotypic variation (Costet et al. 2012). Similarly, another QTL mapping study identified a major QTL associated with yellow leaf spot disease resistance. Additionally, sugarcane mosaic disease (SMD), caused by the sugarcane mosaic virus (SCMV) alone or combined with other viruses, severely impacts yield by reducing photosynthesis. Seven mosaic resistance QTLs were

identified in a QTL mapping study involving 285 F1 progenies from a cross between a moderately resistant and a highly susceptible variety. Furthermore, nine genes with differential expression between resistant and susceptible varieties were found to play a key role in SCMV resistance (Lu et al. 2023). SNP, SSR, and ISSR markers have also been used for QTL mapping of resistance to leaf scald disease (Gutierrez et al. 2018).

12.3.2 Increasing Yield and Sugar Content

Several factors, including stalk girth, stalk height, and the number of millable canes, influence sugarcane yield and sugar content. Regulating these traits involves multiple genes or loci distributed across the genome. Additionally, the timing of sugar accumulation plays a critical role in sugar content, with early maturing varieties generally exhibiting higher sugar content early in the season, which benefits farmers. Identifying molecular markers linked to these agronomic traits significantly reduces the time and cost of developing modern sugarcane varieties by enabling the selection of optimal parental plants for crossing and accelerating genetic gains in breeding programs. Association studies, which identify molecular markers associated with specific traits, have become widely used in various crops (Breseghello and Sorrells 2006; Agrama et al. 2007. The first attempt to map sugar content and yield traits in sugarcane was made by Ming et al. (2001, 2002), who identified 36 marker-trait associations (QTLs) in progeny from interspecific crosses between *S. officinarum* (Green German) and *S. spontaneum* (IND81–146), as well as between *S. spontaneum* (PIN-84-1) and *S. officinarum* (Muntok Java). These studies identified 14 QTLs in the first cross and 22 in the second cross-related to sugar content. Another survey by Hoarau et al. (2002) mapped 45 quantitative trait alleles (QTAs) related to sugar content in the selfed progeny of the R570 cultivar using 1000 AFLP markers. They identified S-QTAs (single-marker QTAs), M-QTAs (multi-marker QTAs), and interaction QTAs (int-QTAs), detecting significant loci linked to stalk length, stalk diameter, number of millable stalks, and Brix. Alwala et al. (2009) also mapped sugar-related traits using AFLP, SRAP, and TRAP markers in a cross between *S. officinarum* (Louisiana stripped) and *S. spontaneum* (SES-147B). They identified 41 putative QTLs, with individual QTLs explaining 15.1–21.6% of the phenotypic variation. Some QTLs were unique to the early plant-growing season, while others were consistent across multiple years.

12.3.3 Enhancing Stress Tolerance

Abiotic stresses such as drought, salinity, and cold significantly impact plant growth and productivity (Swapnil et al. 2021; Meena et al. 2022). These environmental stresses, common in major crops such as wheat, rice, maize, and barley, reduce

sugarcane yield and quality Tailor et al. 2024). Genes involved in stress tolerance, including transcription factors and kinase genes, enable sugarcane plants to withstand these unfavorable conditions. Recent sugarcane genome sequencing and mapping advances have identified molecular markers associated with abiotic stress tolerance. Several studies have used molecular markers such as AFLP, RAPD, and SSR to identify genotypes with resistance to abiotic stresses. For example, RAPD markers have been used to identify drought and salinity-resistant markers in sugarcane.

Khaled et al. (2017) conducted a marker-assisted selection study in eight sugarcane genotypes using RAPD, ISSR, and R-ISSR (a combination of RAPD and ISSR) markers for drought tolerance. Their results showed that R-ISSR markers were more efficient than the other two. Under salt stress, sugarcane exhibits reduced growth, lower sugar content, poor sprout emergence, and decreased productivity. Molecular markers, particularly RAPD, can track loci or genes associated with salt tolerance, reducing the need for time-consuming phenotypic measurements. A study involving in vitro culture of sugarcane embryonic calli treated with ethyl methane sulfonate (EMS) under drought and salt stress conditions identified polymorphisms through RAPD marker analysis, helping to separate stress-tolerant lines from controls. Overall, molecular markers are essential tools for improving sugarcane breeding programs, enabling the introduction of desirable traits related to biotic and abiotic stress responses by identifying genetic and phenotypic diversity.

12.4 Challenges and Limitations of Marker-Assisted Breeding in Sugarcane

Despite the significant potential of molecular markers in improving key traits such as stress tolerance, sugar content, yield, and disease resistance, marker-assisted breeding (MAB) in sugarcane faces several challenges. One major issue is the complex and polyploid genome structure of sugarcane, which complicates genetic analysis and trait inheritance studies. Compared to other crops, the limited number of molecular markers available for sugarcane restricts the ability to cover a wide range of traits efficiently. Moreover, many important characteristics, such as sugar yield and disease resistance, are quantitative and controlled by multiple loci, making identifying specific markers linked to these traits difficult. The high cost and time required for MAB, along with the need for sophisticated laboratory equipment and skilled personnel, represent significant limitations. Regulatory issues also complicate the implementation of MAB, particularly in cases involving genetically modified organisms (GMOs), which could hinder transgenic breeding methods. Despite these challenges, MAB remains a powerful tool for improving sugarcane breeding, particularly when integrated with conventional breeding techniques, and holds great promise for advancing the development of improved sugarcane cultivars with higher yields, disease resistance, and stress tolerance.

12.5 Ethical Considerations in Marker-Assisted Breeding

The ethical discourse surrounding gene manipulation and marker-assisted breeding (MAB) in plant genetics involves environmental concerns and corporate market dynamics. Proponents of gene-edited crops often frame them as a form of "climate-smart" technology, highlighting their potential to address the challenges posed by climate change. These crops are considered resilient to diseases and environmental stressors, and their improved efficiency could help reduce post-harvest waste and lower carbon footprints. Gene-edited plants can also contribute to more sustainable agricultural practices by enhancing crop resilience. However, critics raise significant concerns regarding the safety of gene manipulation techniques, particularly in relation to gene drives. The incomplete understanding of off-target effects associated with gene editing techniques raises questions about their long-term safety, environmental impact, and unintended consequences for biodiversity (Clapp and Ruder 2020; Idris et al. 2023). Furthermore, cultural factors, particularly in regions with traditional solid agricultural practices such as India, can influence the public perception and acceptance of genetic manipulation, adding a layer of complexity to the ethical debate.

From a corporate perspective, reducing regulatory barriers for gene-edited crops has lowered the development costs for these technologies, making it easier for large and small entities to bring gene-edited crops to market. Proponents argue that these relaxed regulations have democratized access to gene-editing technologies, allowing non-corporate players to enter the market. However, critics contend that gene editing may give agricultural biotechnology firms disproportionate control over the food supply chain, potentially increasing their influence over farmers and consumers alike (Hefferon and Herring 2017; Helliwell et al. 2022). This concern highlights a broader issue within the ethical debate about the consolidation of power within the agricultural biotechnology industry and its potential to undermine food sovereignty and farmers' rights.

The ethical considerations of plant breeding extend beyond biosafety concerns to encompass a range of ecological, biochemical, and socio-economic impacts. The introduction of genetically modified (GM) crops may result in unintended environmental consequences, such as disrupting local ecosystems or transferring engineered traits to wild relatives. Additionally, the biochemical changes introduced by new genes can alter the nutritional content or the toxicity of crops in ways that are not immediately evident, potentially affecting food safety and security (Mahmood et al. 2017; Lakra et al. 2019). Ethically sound plant breeding practices should account for the needs of the farmers who cultivate these crops and the consumers who depend on them. The socio-economic implications for farmers, particularly smallholder farmers, also deserve consideration, as adopting new technologies may introduce financial risks, alter market dynamics, or reinforce economic inequalities.

The notion of "naturalness" complicates the ethical discourse surrounding genetic manipulation. Many critics of gene modification argue that GM crops are "unnatural" and, as a result, may be ethically questionable. This view often stems from the belief that artificially altering the genetic makeup of organisms violates natural boundaries and undermines the integrity of living systems. Such ethical concerns are particularly salient in genetic engineering, where crops produced through gene editing may be perceived as more "unnatural" than those developed via conventional breeding practices. Public perception often associates genetically modified organisms (GMOs) with unnaturalness, which shapes the ethical evaluations of these technologies (Gaskell et al. 2011; Mielby et al. 2013). Even genetically validated modifications may be viewed with ethical skepticism if they pose perceived risks to human health, such as introducing allergens or other harmful substances.

Integrating ethical principles into scientific and technological advancements remains a complex and ongoing challenge. Introducing novel biotechnologies often generates tensions with longstanding practices and traditions, especially in agriculture. In countries like India, where multi-cropping systems have been an integral part of agricultural practice, adopting new technologies like marker-assisted breeding or genetic modification may disrupt traditional farming practices, leading to ethical concerns about cultural preservation and farmers' rights (Jamieson 2000).

Various ideological frameworks shape the ethical discourse surrounding genetic modification. Theological perspectives, for example, often view all life as sacred, equating genetic manipulation with "playing God" and thus raising moral objections to such interventions. On the other hand, reductionist views tend to conceptualize life as a collection of genes and argue that gene transfer across species is a natural extension of biological processes. From this standpoint, genetic manipulation may be seen as a normal, even necessary, progression in the evolution of agriculture (Koepsell 2007). Utilitarian ethics, which focuses on maximizing overall benefit and minimizing harm, provides another perspective, suggesting that the potential benefits of genetic engineering—such as increased crop yields, disease resistance, and environmental sustainability—should be weighed against the risks. However, evaluating the broader ecological and socio-economic impacts of genetic modification remains a challenge, as the long-term consequences of these technologies are difficult to predict.

In addition, the ethical considerations surrounding marker-assisted breeding and gene editing in plants are complex and multifaceted. While these technologies offer significant potential for enhancing agricultural sustainability and addressing global food security challenges, they raise critical questions about safety, environmental impact, socio-economic consequences, and cultural implications. As these technologies continue to evolve, engaging in ongoing dialogue among scientists, ethicists, policymakers, and the public is essential to ensure that the development and deployment of genetic technologies align with societal values and ethical principles.

12.6 Future Scope

The integration of multi-omics approaches, including transcriptomics and metabolomics, with genomic data holds immense promise for further improving the understanding of sugarcane's genetic architecture. In particular, developing comprehensive genomic resources, such as high-density SNP arrays and advanced genotyping technologies, will enable more efficient selection and breeding strategies. The application of gene editing technologies, such as CRISPR/Cas9, RNAi in combination with molecular marker-assisted breeding, could further accelerate the development of sugarcane varieties with enhanced traits, including higher yield, better disease resistance, and increased stress tolerance (Das et al. 2011; Verma et al. 2024). Additionally, the ongoing refinement of bioinformatics tools to handle large-scale genomic data will facilitate the more accurate and faster analysis of genetic variation across diverse sugarcane populations. As these technologies evolve, there is a growing opportunity to translate the insights gained from genetic research into commercially viable sugarcane cultivars, thereby contributing to the sustainability and productivity of the global sugarcane industry. However, ethical considerations related to the use of genetic modifications and the need for responsible biotechnology practices must remain a central focus in future breeding efforts.

12.7 Conclusion

Using molecular markers in sugarcane breeding represents a critical advancement in improving traits such as sucrose yield, disease resistance, and stress tolerance. Marker-assisted selection (MAS), combined with advanced genomic techniques, has significantly enhanced the precision and efficiency of breeding programs. The progression from traditional methods to PCR-based and DNA sequence-based markers has provided breeders with an array of powerful tools to dissect the complex genetic makeup of sugarcane, a crop characterized by its polyploidy and high heterozygosity. Although substantial progress has been made in identifying genetic loci associated with desirable traits, challenges remain due to the high cost and technical expertise required for high-throughput sequencing and marker validation. Furthermore, the complexity of sugarcane's genome necessitates the continued development of more accessible, cost-effective, and scalable genomic tools for practical breeding applications.

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Chapter 13
Utilization Technologies of miRNAs for Sugarcane Genetics and Breeding

Garima Aggarwal, A. S. Jeena, Babita Bhatt, Rubina Khan, Divya Chaudhary, Dalip, and Kamal Priyananda Wickramasinghe

Abstract MicroRNAs are tiny, non-coding RNAs that play vital roles in regulating gene expression at the post-transcription level in plants. In sugarcane, a crop of significant economic value for sugar and biofuel production, miRNAs play a vital role in modulating key agronomic and physiological traits. MicroRNAs (miRNAs) are pivotal in gene expression control, influencing plant growth, development, and reactions to environmental pressures. Understanding miRNA biogenesis and regulatory networks opens pathways for precise crop improvement. The application of miRNA-based methods in sugarcane marks a substantial leap forward in crop improvement, enabling precise manipulation of gene expression to enhance desired traits. Scientists have devised robust strategies to improve stress resilience, optimize growth, and increase yields in sugarcane by exploiting miRNA pathways. Each approach—be it transgenesis, target mimicry, or CRISPR-mediated editing—offers distinct benefits, ranging from fine-tuning gene expression to achieving non-transgenic modifications through topical applications. Computational tools and databases further boost these endeavors, facilitating predictive modeling and evolutionary analysis of miRNA-target interactions. Collectively, these techniques and resources constitute a comprehensive toolkit for sugarcane enhancement. In sugarcane, particular miRNAs have demonstrated considerable potential for improving resistance to abiotic stresses—such as drought, salinity, cold, and nutrient deficiencies—and biotic stresses from pathogens and pests. Key miRNAs identified in

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sugarcane's response to abiotic stress include ssp-miR164 (targeting NAC transcription factors under drought conditions), miR159 (MYB protein regulation during salinity stress), and miR319 (cold response modulation). Furthermore, miRNAs such as miR156 and miR444 have increased resilience to nutrient limitations, illustrating the extent of miRNA involvement in abiotic stress responses. Biotic stress tolerance is similarly enhanced by miRNAs such as miR160 (targeting SCMV resistance pathways), sof-miR159c (regulating viral helicase ORF of SCMV), and miR444a (associated with defenses against fungal pathogens). These miRNAs, through their specific regulatory targets, activate pathways that enhance sugarcane against a range of stressors, highlighting the potential of miRNA-based genetic approaches to strengthen crop resilience. Ongoing investigation of miRNA-based technologies in sugarcane presents promising avenues for breeding resilient varieties. These discoveries open up new possibilities for sugarcane improvement, using miRNAs to enhance the plant's resilience and productivity.

Keywords microRNAs · Biogenesis · Regulation · Sugarcane · Sstress tolerance · Biotic and abiotic stresses · miRNA techniques

13.1 Introduction

Sugarcane (*Saccharum* spp.), a vital crop in the global agricultural industry, is taxonomically assigned to the Poaceae family, Panicoideae subfamily, and Andropogoneae tribe. This tribe primarily comprises polyploid, perennial species utilizing C_4 photosynthesis (Verma et al. 2020a, 2024a, b). Hybrid sugarcane varieties are important in agriculture, being grown in over 80 countries across tropical and subtropical regions (Singh et al. 2019; Chen et al. 2020; Li et al. 2024). As one of the world's primary industrial crops, sugarcane originated in southern Asia, particularly India, China, and New Guinea, with cultivation dating back roughly 10,000 years (Zhang et al. 2018; Liang et al. 2024). Renowned for its exceptional photosynthetic efficiency, sugarcane is pivotal in global sugar production (Verma et al. 2022a, b, 2023a). Its unique ability to accumulate sugar in parenchymatous tissues at remarkably high concentrations distinguishes it from other crops. Furthermore, its substantial biomass content makes it an excellent source of feedstock for sustainable renewable energy production (Swapna and Kumar 2017; Singh et al. 2020; Malviya et al. 2022). Sugarcane's utility extends far beyond sugar production. Its ethanol yield surpasses that of maize by a factor of five. Furthermore, the crop generates valuable chemicals, while its byproducts are used extensively in ethanol production, animal feed, cultivation substrates, and power generation (Huang et al. 2020; Song et al. 2019, 2022).

Despite its vital role in global sugar production and other valuable applications, sugarcane breeding faces considerable challenges. While sugarcane accounts for

80% of the world's sugar supply, efforts to improve yields have plateaued due to limited genetic diversity, extended breeding cycles, and the intricacy of its polyploid genome (Wu et al. 2022; Guo et al. 2023; Bhatt et al. 2024). This complexity, resulting from a breeding history characterized by hybridization and backcrossing, has led to intricate genome structures. These include mixed subgenomes, recombined chromosomes, and regions with highly variable gene pairing, hindering modern molecular breeding approaches (Cursi et al. 2022; Lu et al. 2024; Afghan et al. 2024). A significant advancement was achieved by Healey et al. (2024) with the development of a reference genome for the R570 sugarcane cultivar. This genome assembly provides valuable insights into sugarcane's genomic structure, particularly in recombination, aneuploidy, and chromosome organization. This reference genome will significantly assist future molecular breeding efforts by enabling more precise genetic improvements.

Sugarcane genome is highly polyploid, comprising approximately 12 chromosome copies derived from various species. The modern cultivar R570 originated from hybridization between the domesticated *Saccharum officinarum* and the wild *Saccharum spontaneum*. Early hybrid generations exhibited lower sugar yields, but backcrossing with *S. officinarum* helped restore high sugar content. Conventional breeding remains crucial to sugarcane improvement but encounters challenges due to the crop's polyploid genome, which complicates trait inheritance and genetic uniformity. A significant challenge is the limited genetic diversity in modern sugarcane varieties, with 78–80% of their genome derived from *S. officinarum* and only 10–20% from *S. spontaneum,* which restricts genetic variability and cultivar enhancement (Piperidis et al. 2010; Li et al. 2023; Huang et al. 2024).

Moreover, environmental factors and genotype–environment interactions make it difficult to predict the performance of new cultivars, necessitating extensive trials for trait stability. The breeding process is also lengthy, often exceeding a decade, which further constrains the potential for enhancement (Verma et al. 2019a, b; Chen et al. 2024). To overcome these challenges, molecular techniques have been integrated into breeding programs, offering new possibilities for genetic improvement. Recent developments in transcriptomic and proteomic methods have shed light on the molecular foundations of sugar production and stress resilience in sugarcane, uncovering crucial molecular mechanisms that influence the crop's sensitivity and tolerance, resulting in productivity improvements (Barnabas et al. 2016; Malviya et al. 2020; Singh et al. 2021a, b, 2022). The transcriptome comprises all RNA transcripts within a cell under specific conditions, and transcriptomic sequencing has broadened our knowledge of RNA molecules, including mRNA, tRNA, rRNA, and non-coding RNA. In recent years, miRNA-based techniques have emerged as potent tools for crop enhancement, allowing precise regulation of gene expression to improve desirable traits (Singh et al. 2021; Guo et al. 2021, 2022). As sequencing technologies have advanced, microRNAs (miRNAs) have become valuable molecular instruments for sugarcane breeding.

miRNAs are small, non-coding RNAs that control gene expression and are involved in essential biological processes such as stress responses, growth, and metabolism. miRNAs offer promising approaches for addressing yield, disease

resistance, abiotic stress tolerance, and sucrose accumulation in sugarcane (Verma et al. 2020b, c; Sharma et al. 2021; Tian et al. 2023; Chen et al. 2023). This complex polyploid crop is crucial for global sugar and biofuel production. As sugarcane faces biotic and abiotic stresses exacerbated by climate change, miRNA-based techniques offer a promising approach to enhance crop resilience and productivity (Verma et al. 2020d, e; Anas et al. 2021a, b; Malviya et al. 2024). By manipulating miRNA pathways through transgenesis, target mimicry, CRISPR, and other innovative methods, researchers can precisely influence gene expression, targeting specific stress tolerance mechanisms and growth traits (Malviya et al. 2021; Oliveira et al. 2022). This chapter examines the function of miRNAs in sugarcane biology and outlines a comprehensive framework of miRNA techniques for sugarcane.

MicroRNAs (miRNAs) have become key regulators of gene expression across diverse biological processes, including growth, development, stress response, and environmental adaptation, making them valuable tools for crop improvement. Understanding the biogenesis of miRNAs is crucial, as it reveals pathways to modulate miRNA levels and thus influence specific plant traits. Recent advances have introduced miRNA-based techniques to enhance agronomic traits in crops such as sugarcane, which holds significant value for biofuel, sugar production, and various industrial applications (Joshi et al. 2022, 2023; Xu et al. 2022, 2024). Techniques such as transgenesis, artificial miRNA (amiRNA) technology, target mimicry, and CRISPR-based genome editing allow for precise modulation of miRNA pathways. By adjusting miRNA levels or inhibiting particular miRNA interactions, researchers can improve stress tolerance, increase yield, and optimize growth traits in sugarcane. This chapter explores the current miRNA-based approaches in plant research, highlighting practical applications, methodological innovations, studies, and associated challenges in sugarcane crop.

13.2 The Process of miRNA Biogenesis

MicroRNAs (miRNAs) are compact (21–24 nucleotide), endogenous, non-coding RNA sequences located in intergenic genomic areas. These molecules are instrumental in post-transcriptional gene regulation, exerting significant influence on gene expression in plants at both transcriptional and post-transcriptional levels (Morris and Mattick 2014). By binding to target messenger RNAs (mRNAs), miRNAs can either induce mRNA degradation or suppress translation, thus providing a robust regulatory mechanism for diverse biological processes, including plant growth, development, and stress responses. In sugarcane, miRNAs control essential traits, such as drought tolerance, disease resistance, and biomass production, making them valuable targets for genetic improvement (Gentile et al. 2015; Silva et al. 2021; Krishna et al. 2022; Zheng et al. 2023).

The formation of miRNAs in plants involves a series of precisely coordinated steps (Fig. 13.1), beginning with the transcription of miRNA genes (MIR genes) by RNA polymerase II (Pol II), which produces primary miRNA transcripts

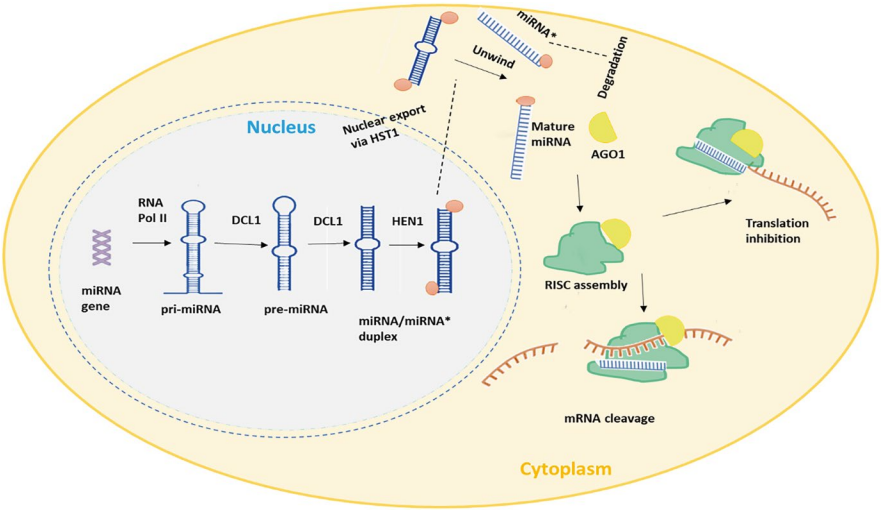

Fig. 13.1 Overview of miRNA biogenesis pathway

(pri-miRNAs) (Rogers and Chen 2013). These pri-miRNAs undergo capping and polyadenylation to ensure stability and proper processing. Transcriptional regulation is influenced by cis-regulatory elements and interactions with transcriptional coactivators such as Mediator, facilitating Pol II recruitment to MIR promoters. The pri-miRNAs adopt stem-loop structures and are processed by a multi-protein complex, including DICER-LIKE1 (DCL1), HYPONASTIC LEAVES1 (HYL1), and SERRATE (SE). These proteins cleave pri-miRNAs near the stem base, yielding precursor miRNAs (pre-miRNAs). Structural features within the pri-miRNA, such as a 14- to 15-base-pair stem, guide accurate cleavage by DCL1 and ensure efficient processing (Rhoades et al. 2006).

In plants, DCL1 and associated factors concentrate in specialized subnuclear regions known as dicing bodies, where miRNA processing occurs. Factors such as MOS2 regulate the formation and abundance of dicing bodies by influencing the recruitment of the DCL1 complex to pri-miRNAs. After dicing, the resulting miRNA/miRNA* duplex undergoes 3′-end methylation by HUA ENHANCER1 (HEN1) to prevent degradation. This methylation is essential for stabilizing miRNAs in vivo. The duplex then dissociates, and the miRNA guide strand is incorporated into the RNA-induced silencing complex (RISC), which is primarily bound by ARGONAUTE1 (AGO1) for target gene silencing (Zhang et al. 2018). Following processing, mature miRNAs are exported to the cytoplasm by the HASTY (HST) protein, where they are loaded into AGO proteins within the RISC complex. This step depends on sequence features such as the 5′ nucleotide, influencing AGO loading preferences. Once loaded, miRNA-bound AGO directs the precise targeting of complementary mRNA transcripts for gene silencing through slicing or translational repression (Pomeranz et al. 2010).

miRNA targets are specific mRNA molecules that microRNAs (miRNAs) bind to regulate gene expression, usually by either degrading the mRNA or repressing its translation. Predicting miRNA targets is a sophisticated process that relies on computational and experimental approaches. These include sequence complementarity analysis, where tools look for matches in the "seed" region of the miRNA (the initial nucleotides responsible for binding specificity), as well as assessments of mRNA secondary structure and target site accessibility. Prediction tools such as psRNATarget, miRBase, and TargetScan, incorporate these factors to identify potential target sites. At the same time, experimental methods such as Degradome-Seq, qRT-PCR, and reporter assays help validate these predictions in a biological context. The stability of miRNAs is maintained through 2'-O-methylation, although they are ultimately degraded by enzymes, such as SMALL RNA DEGRADING NUCLEASE 1 (SDN1), which modulates miRNA levels to ensure timely turnover. Recent investigations reveal that miRNAs regulate various biological processes, from plant growth and development to physiological responses under biotic and abiotic stresses (Song et al. 2021; Huang et al. 2022; Bhatt et al. 2023a, b).

13.3 Harnessing MicroRNAs and Cutting-Edge Biotechnological Tools for Crop Improvement

MicroRNAs regulate target mRNA levels and timing, indirectly affecting various plant functions. Many transcription factors controlled by other miRNAs can initiate the transcriptional regulation of miRNA-producing genes. Fine-tuning MIR genes is a potent biotechnological approach to enhance abiotic or biotic stress resistance in economically significant crops (Sharma et al. 2022; Kumari et al. 2022, 2023). Our understanding of MIR gene complexity has improved due to advancements in next-generation sequencing (NGS), degradome sequencing technologies, bioinformatics tools, open-access miRNA databases, and stem-loop real-time RT-PCR. Notably, overexpressing specific MIR genes, whether continuously in particular tissues or triggered by stress or ageing, has improved desired agronomic traits across various plant species (Solanki et al. 2023; Singh et al. 2023). Current miRNA fine-tuning strategies involve using genetic engineering techniques to manipulate the final concentration of these miRNAs in the cytoplasm and modulate MIR gene transcription. The successful biotechnological methods explored for MIR gene or miRNA modification have improved agronomic characteristics in several model or crop plants.

13.3.1 Genetic Engineering Techniques to Enhance miRNA Functionality

13.3.1.1 Transgenesis, Cisgenesis, and Intragenesis

These methods involve introducing foreign genes (transgenesis) or native genes (cisgenesis) into a plant's genome to modify the expression of microRNAs (miRNAs) that govern stress responses and development. By inserting these MIR genes, plants can be engineered to show improved resistance to biotic (e.g., pests, pathogens) and abiotic stresses (e.g., drought, salinity). Transgenesis allows precise manipulation of specific gene functions by increasing or decreasing miRNA levels, potentially enhancing traits such as disease resistance, yield, or stress tolerance (Deng et al. 2023; Jagadesh et al. 2024). Intragenesis is an innovative genetic engineering technique to improve crop characteristics using genetic material from the same or closely related species. The transfer of one or more MIR genes between non-crossable plant species (transgenesis) or between crossable or the same species (cisgenesis and intragenesis) has been successfully achieved in numerous plant species. Additionally, any part of the T-DNA can theoretically be modified to boost transgenic expression, or the original native sequence can be used to maintain cisgenic properties.

Overexpression methods are also employed, introducing additional copies of miRNA genes or synthetic analogues into plants to increase miRNA levels and examine the effects on target genes. Techniques such as transgenic overexpression, where miRNA genes are inserted into the plant genome under strong promoters, enable stable, long-term enhancement of miRNA levels. Viral vectors can induce overexpression for temporary studies without creating permanent transgenic lines. Overexpression approaches allow scientists to evaluate miRNA roles in specific traits, such as drought tolerance or stress response, by observing the phenotypic outcomes of suppressed target gene expression. However, these methods can result in unintended effects and require careful validation to avoid unexpected phenotypic changes. These techniques provide a comprehensive toolkit for analyzing miRNA functions, offering profound insights into the regulatory networks that control plant development and stress resilience.

13.3.1.2 Artificial MIR Gene

Currently, numerous approaches exploit post-transcriptional gene silencing (PTGS) to inhibit or investigate specific gene functions. These methods primarily rely on the continuous overproduction of a modified target gene (complete or partial) in either antisense or sense/antisense orientation, as well as the generation of small interfering RNAs (siRNAs) from double-stranded RNAs (dsRNAs) using a virus-induced

gene silencing (VIGS) model. However, these techniques produce various siRNAs that may inadvertently silence unintended genes, resulting in off-target effects. To address this issue, an artificial microRNA (amiRNA)-based approach was developed to effectively silence target genes and produce specific miRNAs (Zhang et al. 2018). Artificial miRNAs are engineered RNA molecules designed to specifically target and silence one or more plant genes, imitating natural miRNAs but offering greater precision.

These molecules are crafted to bind to complementary mRNA sequences, enabling targeted gene silencing. amiRNA provides high accuracy in gene regulation, minimizing unintended effects and unwanted changes in non-target pathways. These amiRNAs maintain a conserved secondary foldback structure, similar to a typical pre-miRNA. However, the original miRNA-5p: miRNA-3p sequence is replaced with a synthetic miRNA targeting a specific mRNA. Consequently, amiRNAs can be engineered to target any mRNA more precisely than methods relying on siRNA accumulation or dsRNA overexpression. Pre-amiRNA processing typically generates a single amiRNA targeting a specified sequence to reduce off-target effects. The amiRNA method has shown effectiveness in gene knockout experiments in controlling endogenous gene expression and exploring novel MIR gene functions (Wagaba et al. 2016).

13.3.2 Target Mimicry for Controlled miRNA Regulation

13.3.2.1 Endogenous and Artificial Target Mimicry

Target mimicry for controlled miRNA regulation: Unlike MIR gene overexpression, which increases miRNA levels, endogenous and artificial target mimics reduce specific miRNA activity to achieve desirable agronomic traits. Lin et al. (2015) reported that endogenous target mimicry (eTMs) is a natural mechanism regulating miRNA accumulation and controlling various plant biological processes. eTMs comprise numerous circular noncoding RNAs (circRNAs) or long noncoding RNAs (lncRNAs) transcribed from the genome, typically expressed differently in response to stressors or unfavorable conditions. Artificial target mimicry involves designing sequences resembling miRNA target sites that bind to specific miRNAs, effectively sequestering them and diminishing their activity. This prevents miRNAs from interacting with their natural target mRNAs, allowing those genes to be expressed commonly. Target mimicry is reversible and can be fine-tuned, providing control over miRNA regulation without permanent genomic alterations.

13.3.3 Genome Editing Approaches for miRNA Modification

13.3.3.1 MIR Gene Editing Using Endonucleases and CRISPR Systems

Plant genome editing initially utilized meganucleases and zinc-finger proteins (ZNFs). Meganucleases have limited target options due to their high specificity in recognizing 12–42 nucleotide double-strand DNA sequences. ZNFs consist of two tandem repeat DNA-binding domain modules surrounding the FokI nuclease catalytic domain (Osakabe and Osakabe 2015). Each module is specific to ≥24 nucleotides, with each domain recognizing a distinct nucleotide triplet. Transcription activator-like effector nucleases (TALENs) are similarly structured, with two tandem repeat DNA-binding motif modules flanking a FokI motif. Each TALEN DNA-binding motif identifies a single nucleotide. Recently, RNA-guided nuclease systems such as CRISPR/Cas9, CRISPR/Cpf1, and CRISPR/Csm1 have been adapted for plant genome editing (Wang et al. 2018).

These genome editing technologies, particularly CRISPR systems, allow for precise MIR gene modification or deletion to alter or eliminate specific miRNA functions. While CRISPR/Cas9 is most commonly used, Cpf1 and Cas13a systems offer additional target range and mutation pattern options. Both nucleases create double-strand breaks (DSBs) at the target site, potentially resulting in nucleotide insertions or deletions (indels) during repair. Targeted nucleotide editing can also be achieved using engineered donor DNA. CRISPR/Cas9 nonhomologous end joining (NHEJ) can introduce indels to pre-miRNA sequences or MIR gene miRNA processing sites, inhibiting or delaying miRNA production (Zhou et al. 2017). Indel insertion in target genes can disrupt miRNA-target mRNA pairing, preventing mRNA cleavage into RISC. Moreover, complete deletion or knock-in of MIR genes or their promoter regions can be accomplished through homology-directed repair (HDR) and homology and recombination-directed repair (HRDR) (Zhao et al. 2016). In transgene-free CRISPR/Cas9 or Cpf1 systems, nuclease and gRNA are produced in vitro and introduced into plant cells via biolistics (Liang et al. 2017). CRISPR-based gene editing offers precise, efficient, and heritable MIR gene alterations, enabling stable modifications that enhance stress tolerance without introducing foreign DNA.

13.3.4 Topical Delivery of Pri-miRNA or Mature miRNA

RNA interference (RNAi) technology has been refined for topical delivery or foliar spray of nanostructured and stabilized dsRNA molecules in model or crop plants, targeting pathogen control and insect pest management without using transgenes (McLoughlin et al. 2018). Topical delivery involves directly applying synthetic miRNAs or miRNA precursors to plant tissues, allowing these molecules to temporarily enter plant cells and adjust miRNA levels without genetic modification,

resulting in transient gene expression changes. Various carrier nanoparticles have been successfully optimized to enhance the delivery and internalization of highly integrated RNA within plant cells. These include biopolymers such as chitosan, silicon, carbon, and clay nanosheets; ribonucleoprotein particles such as peptide transduction domain–dsRNA binding domain; and cross-linkers such as tripolyphosphate, dextran sulphate, and poly-D-glutamic acid (Cunningham et al. 2018).

A significant advantage of this approach is its non-GMO method for altering miRNA levels, potentially making it more favorable from regulatory and consumer standpoints. This technique is also highly versatile for research and experimental applications. However, the technology is still in development for plant use to deliver pri-miRNA or mature miRNA, aiming to modify native genes to improve agronomic traits or to combat insect pests or diseases.

13.3.5 Epigenetic Memory and miRNA-Mediated Gene Regulation

13.3.5.1 Epigenetic Memory

Plants frequently encounter multiple stressors simultaneously, triggering defense mechanisms that mitigate the adverse effects of other stressors. These defense responses are enhanced through epigenetic mechanisms, which preserve "molecular memories" of previous stressors. This epigenetic memory enables more efficient defense or adaptive responses when the same stress recurs. The primary epigenetic modifications in genomic regions containing regulatory sequences, protein-coding genes, or MIR genes include histone alterations (such as methylation, phosphorylation, acetylation, ubiquitylation, and sumoylation) and siRNA or miRNA-directed DNA methylation (Xie and Yu 2015). Epigenetic memory utilizes miRNAs to guide DNA methylation and histone modifications, resulting in stable gene expression changes without altering the underlying DNA sequence. This method relies on miRNA-induced transcriptional gene silencing to produce long-lasting effects. It generates inheritable modifications not involving DNA sequence changes, potentially simplifying regulatory approval processes for non-GMO crops. Crisp et al. (2016) note that 24-nt miRNAs are the primary mechanism for epigenetic genome modification, affecting the expression of numerous genes or MIR genes involved in various biological processes. Consequently, the transcription of many MIR genes and eTMs implicated in stress response networks is indirectly suppressed or stimulated by epigenetic changes in the genome triggered by biotic or abiotic stress events.

13.4 Functional and Phylogenetic Analysis of miRNA in Sugarcane

13.4.1 Degradome in miRNA Functional Analysis

Degradome analysis is a powerful technique for identifying and validating miRNA targets by analyzing the mRNA degradation products generated when miRNAs bind to their targets and induce cleavage. This method captures the fragments of mRNAs produced during cleavage, providing direct evidence of miRNA-mediated degradation and confirming computationally predicted targets. By mapping these cleavage sites, degradome sequencing offers valuable insights into miRNA-target interactions and contributes to the understanding of miRNA networks involved in processes such as stress tolerance and developmental regulation. The analysis begins with RNA extraction from plant tissues (e.g., roots, leaves) exposed to specific treatments such as salt stress.

After RNA quality assessment, small RNAs (18–30 nt) are isolated, and adapter sequences are added to prepare the samples for high-throughput sequencing (e.g., Illumina HiSeq). Raw reads are processed through cleaning, trimming, and alignment to the reference genome. Degradome tags, which indicate miRNA-guided mRNA cleavage, are mapped to miRNA sequences at the 10th or 11th nucleotide position. Targets are then classified, and Gene Ontology (GO) analysis helps predict their biological functions. To validate the identified miRNA-target interactions, quantitative real-time PCR (qRT-PCR) is employed to compare expression levels between treated and control samples. Statistical analyses, such as t-tests, identify significant expression changes, and results are visualized through T-plots or miRNA-target interaction networks. This approach enhances the understanding of miRNA regulation and target gene networks, particularly under stress conditions (Liu et al. 2022).

13.4.2 Dendrogram Sequencing (Phylogenetic Analysis)

Dendrograms, or phylogenetic trees, are visual representations based on sequence similarities that illustrate evolutionary relationships between genes or miRNAs. These diagrams aid in identifying conserved miRNAs across species by grouping similar sequences, offering insights into evolutionarily conserved functions and potential regulatory pathways in plants. They facilitate the identification of functionally identical or ancestral miRNAs, helping to understand miRNA roles across species. Software such as MEGA (molecular evolutionary genetics analysis), ClustalW, and PHYLIP (phylogeny inference package) align miRNA sequences to create phylogenetic trees that demonstrate evolutionary relationships and conservation patterns.

Computational Target Prediction and GO Enrichment Analysis: Computational studies supplement these experimental approaches by offering bioinformatics tools that predict miRNA targets, examine structural and sequence data, and perform functional classification through gene ontology (GO) enrichment. Tools, such as miRanda, psRNATarget, and RNAhybrid, predict interactions between miRNAs and mRNA target sequences, focusing on complementarity and energy metrics. Bioinformatics tools, such as BLAST and ClustalW, enable scientists to examine miRNA sequence conservation across species. At the same time, GO enrichment platforms such as agriGO and REVIGO classify miRNA target genes into functional groups, enhancing our understanding of their broader biological effects. miRNA target genes are typically found in the 3' UTR of mRNAs. Software like BLAST2GO extracts these sequences, allowing for accurate prediction of miRNA binding sites.

Tools such as miRanda and Spearman's correlation analysis are employed to forecast miRNA–mRNA interactions. These tools verify target relationships and confirm predicted miRNA functions by correlating miRNA and mRNA expression data. This approach provides precise prediction and validation of miRNA targets, aiding in identifying miRNAs that control key genes involved in growth and stress responses. Computational methods facilitate in silico exploration of miRNA networks by constructing predictive frameworks and functional insights, guiding hypotheses before laboratory validation. GO enrichment assesses biological processes, molecular functions, and cellular components linked to predicted miRNA targets, offering insights into these miRNAs' roles in plants. By analyzing the functional categories of miRNA targets, researchers can infer the overarching biological processes that miRNAs may regulate, such as growth, stress response, and metabolic processes.

13.5 Validation Techniques for miRNA Expression and Function

13.5.1 Quantitative Reverse Transcription PCR (RT-qPCR)

This method quantifies miRNA expression levels in real time by converting RNA to cDNA and amplifying specific miRNA sequences. It validates miRNA expression levels identified through high-throughput sequencing, assessing miRNA activity under specific conditions such as stress, hormonal treatments, or developmental stages. The process begins with miRNA isolation from plant tissue, ensuring high-quality RNA extraction. The isolated RNA is then reverse transcribed into cDNA using reverse transcriptase. This cDNA is subsequently amplified using real-time PCR, enabling precise quantification. Fluorescent markers monitor cDNA quantity in real-time during amplification, providing accurate and dynamic measurements of

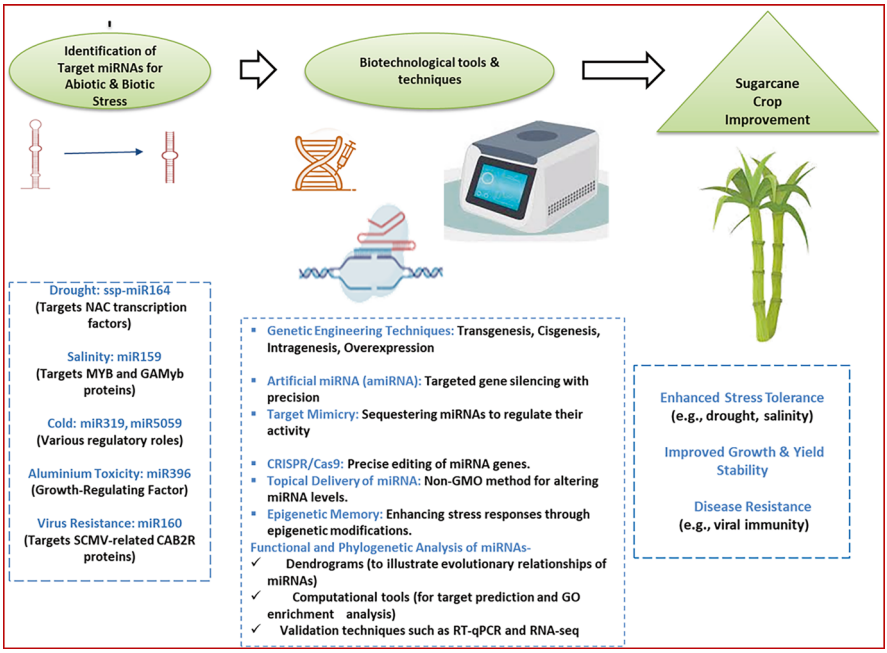

Fig. 13.2 Utilization of MicroRNAs and other miRNA-based techniques for sugarcane crop improvement

miRNA expression levels. RT-qPCR is a highly accurate, quantitative, and reliable technique for validating miRNA expression levels across different samples or treatments.The overall process of sugarcane crop improvement through biotechnological tools is illustrated in Fig. 13.2.

13.5.2 RNA Sequencing (RNA-Seq) for Transcriptome Profiling

RNA-seq generates comprehensive transcriptome data, allowing researchers to identify differentially expressed miRNAs under various conditions, such as internode elongation stages in sugarcane. These sequencing technologies reveal miRNA expression profiles in response to hormonal treatments (such as GA3 and paclobutrazol), which is crucial for understanding miRNA roles in plant stress responses and growth regulation. In sugarcane studies, RNA-seq captures miRNA profiles across developmental stages, contributing to databases that link specific miRNAs with growth traits like stem elongation.

13.5.3 Specialized Databases and Bioinformatics Tools in miRNA Research

13.5.3.1 Micro RNA Databases and Prediction Frameworks

The Plant MicroRNA Database (PMRD) is a plant-specific resource that consolidates all available information on plant miRNAs, such as miRNA sequences, targets, expression data, secondary structures, and genome browse r tools (Zhang et al. 2009). While miRbase hosts many miRNA sequences, PMRD offers a more focused collection specific to plants.

Currently, it encompasses 8433 miRNA sequences from 121 plant species collected through experimental and computational methods (Goswami and Sanan-Mishra 2022). Another comprehensive database, MiRNEST, offers information on miRNAs from viruses, plants, and animals. It amalgamates data from various databases and scientific publications and computational predictions of new miRNAs, their targets, mirtrons, and gene architectures (de Amorim et al. 2022). The database also incorporates degradome data (Volná et al. 2022). Mhuantong and Wichadakul (2009) developed MicroPC, an online tool for predicting and comparing plant miRNAs. This tool employs homology-based analysis to identify conserved plant miRNAs, providing additional insights into their conservation, evolution, and function. Currently, this resource contains 2995 protein targets from 125 plant species across 128 miRNA families. In contrast, the more extensive miRBase database includes miRNA sequences and annotations from various taxa, including plants, mammals, and viruses (Griffiths-Jones et al. 2007).

Specialized databases, such as MicroSugar for sugarcane, consolidate genomic and transcriptomic data to predict miRNAs and their mRNA targets, offering valuable resources for miRNA-based research. MicroSugar utilizes a framework to integrate miRNA sequences, structures, and target predictions specific to sugarcane, providing comprehensive datasets for researchers studying miRNA interactions in plant growth and development. The database features a user-friendly, responsive interface that enables efficient browsing, searching, and downloading of nucleotide sequences, expression data, and miRNA targets. This resource enhances our understanding of miRNA's role in sugarcane development and growth, potentially benefiting sugarcane research.

Qiu et al. (2022) established MicroSugar, a database for annotating mRNA and miRNA in sugarcane (http://suc.gene-db.com). This extensive resource encompasses 194,528 genes, including 80,746 unigenes derived from the Pacbio platform's long reads and 468 miRNAs from 72 samples. The study focused on internode elongation (jointing), a crucial biological process in transforming sugarcane tillers into stems. Combining sequencing data from various stem and tiller internode elongation stages, the researchers extracted 14,300 3′ untranslated region (UTR) sequences from gene sequences. Using the miRanda algorithm and Spearman's Rho of expression levels, they identified 3019 mRNAs as targets for 327 miRNAs. Gene

ontology enrichment analysis was conducted to determine the functions of genes regulated by these miRNAs.

The results revealed that over-represented Gene Ontology (GO) terms were linked to organism formation, suggesting miRNAs play a role in controlling sugarcane growth. These methodologies collectively form a comprehensive toolkit for miRNA research, enabling precise identification, validation, and manipulation of miRNA functions. Each approach, ranging from phylogenetic analysis and quantitative validation to in silico predictions and overexpression, works synergistically, illuminating the intricate regulatory networks governed by miRNAs within plant systems. Through these techniques, researchers have examined the regulatory actions of miRNAs in sugarcane growth and development under stress conditions. This robust framework of techniques has also supported the investigation of miRNAs' roles in sugarcane response and identifying key miRNAs involved in abiotic and biotic stresses.

13.6 MicroRNA-Mediated Regulation in Sugarcane Development and Stress Response

MicroRNAs (miRNAs) have become crucial regulatory elements in sugarcane, significantly influencing traits linked to biomass growth and sugar metabolism. As sugarcane is increasingly recognized as an efficient feedstock for cellulosic bioethanol, the study of miRNAs involved in lignin biosynthesis, cell wall modification, and carbohydrate metabolism has gained prominence.

In biomass accumulation, miRNAs such as miR156 and miR159 have been identified as central players. Studies by Trumbo et al. (2015) have highlighted their roles in recalcitrance (resistance to degradation), bioconfinement, and abiotic stress responses in biofuel crops such as switchgrass and sorghum, which share similarities with sugarcane. miR156, in particular, has garnered attention for its ability to reduce lignin content by as much as 30% in switchgrass. This trait could potentially enhance the efficiency of bioethanol production in sugarcane. Overexpression of miR156 not only impacts lignin but also regulates Trehalose-6-Phosphate Synthase-1 (TPS1), an enzyme crucial for sucrose metabolism, thereby linking biomass production with sugar accumulation (Xie et al. 2014). In addition to miR156, several other miRNAs—miR164, miR166, miR172, and miR398—have been implicated in biomass-related processes.

miR172 works with miR156, balancing the juvenile-to-adult transition and influencing plant architecture, a key determinant of biomass yield (Trumbo et al. 2015). miR166 and miR169 have been linked to shoot development, flower development, and phase transitions, which affect biomass accumulation and sugar production. miR139, identified by Ortiz-Morea et al. (2013), is believed to regulate vegetative bud growth by modulating abscisic acid signaling, a process critical for vegetative biomass accumulation. This miRNA was found to be part of a molecular switch that

controls the activity of dormant buds, with its higher expression in actively growing axillary buds suggesting a role in biomass expansion through vegetative growth. In the context of cell wall formation and modification, 39 miRNAs have been identified in sugarcane roots, particularly during aerenchyma development. Scientists hypothesize that miR156 may act as a negative modulator of pectin breakdown by targeting β-galactosidase in this process.

Moreover, miR172 is thought to target transcripts associated with ethylene-mediated transcriptional activity, indicating its potential involvement in this critical developmental process (Queiroz et al. 2020). Although miRNAs involved in C4 photosynthesis lack direct targets, they are believed to indirectly impact C4 gene expression by acting on transcription factors. Notably, the miR171 family contains putative target genes that could potentially regulate C4 pathway-related genes. These targets, classified as transcription factors (TFs), can influence the expression of other genes, suggesting that sugarcane miRNAs likely participate in the post-transcriptional regulation of chlorophyll synthesis (Hua et al. 2024). However, further experimental validation is required to confirm these interactions. In particular, microRNA 396 has been demonstrated to suppress GROWTH-REGULATING FACTOR (GRF), thereby limiting meristematic activity and promoting earlier sucrose accumulation (Banerjee et al. 2022).

Although no miRNAs have been directly associated with sugar metabolism in sugarcane to date, studies in related biofuel crops offer promising insights. In switchgrass, miR156 and miR139 have been shown to regulate enzymes such as sucrose synthase and TPS1, both essential for sugar metabolism (Xie et al. 2014). The regulatory influence of miRNAs on sugar metabolism is also observed in sorghum, another biofuel crop closely related to sugarcane. Research by Calviño et al. (2011) and Yu et al. (2015) highlighted the significance of miR169 in sorghum sugar accumulation. They observed that three miR169 genes—miR169a, miR169b, and miR169c—exhibit differential expression in the stems and leaves of sweet sorghum, a variety renowned for its high-sugar content. These observations indicate that miRNAs govern both source (leaf) and sink (stem) tissues, thus influencing the overall sugar accumulation process. Furthermore, the miR395/miR395* duplex was equally prevalent in sweet sorghum stem tissues, with Calviño et al. (2011) suggesting a regulatory function for the less abundant miR395 strand in sugar metabolism. Given the genetic similarities between sorghum and sugarcane, it is likely that miRNAs regulating sugar metabolism in sorghum may play similar roles in sugarcane. Wang et al. (2022) pioneered the reporting of miRNAs in sugarcane associated with sucrose accumulation. Through a comprehensive analysis of transcriptome and miRNA data, they identified differentially expressed miRNA-target mRNA pairs involved in sugar metabolism. Of these, 46 targets were identified as transcription factors. Notably, miR172, miR164, miR396, and miR169 were found to regulate transcription factor families such as AP2/ERF, NAC, GRF, and bZIP, which are linked to sugar metabolism.

miRNAs are also heavily involved in stress responses, which can indirectly influence biomass and sugar yield by affecting plant growth and metabolic balance. In biofuel crops, miRNAs such as miR164, miR166, miR398, and miR528 have been

linked to various stress responses, including drought, heat, and salinity tolerance (Trumbo et al. 2015). miR398, in particular, targets genes involved in oxidative stress responses, helping plants mitigate the effects of environmental stressors that could otherwise impede growth and sugar accumulation. Ortiz-Morea et al. (2013) uncovered 26 conserved miRNA families in sugarcane, including miR139 and miR169, which are thought to regulate stress-responsive pathways. Their expression in developing vegetative buds indicates a role in balancing growth and stress response, mainly through abscisic acid signaling. This balance is crucial for sugarcane, as stress tolerance can significantly impact its productivity and biomass yield. The regulatory functions of miRNAs in biofuel crops, such as sugarcane, present opportunities for biotechnological applications to enhance biomass and sugar production. For instance, the overexpression of miR156 has shown potential in reducing lignin content in switchgrass (Trumbo et al. 2015), and similar approaches could be applied to sugarcane to improve bioethanol conversion efficiency.

Furthermore, miRNAs could be targeted to boost sugar accumulation, especially through manipulating miR169, which is associated with sugar metabolism in sorghum and may have a comparable role in sugarcane. However, caution is necessary when considering these applications, as the regulatory networks involving miRNAs are intricate, and miRNA-mediated regulation of target genes may not always yield straightforward results. For example, differential gene expression does not always show a linear correlation with miRNA levels, as observed in sweet sorghum (Calviño et al. 2011).

First to identify miR172, miR164, miR396, and miR169 as significant regulators of sugar metabolism in sugarcane, specifically targeting genes associated with sucrose accumulation—a key goal in sugarcane improvement (Wang et al. 2022). These miRNAs regulate essential genes that produce proteins such as cell wall invertase, glucosidase, hexose carriers, sucrose transporters, UDP-sugar pyrophosphorylase, and soluble acid invertase. Additionally, miR162 has been linked to increased sucrose levels. At the same time, newly identified microRNAs, such as sof-novel1, are involved in regulating genes such as ubiquitin carboxyl-terminal hydrolase (UCTH) and peroxidase, further influencing sucrose storage (Banerjee et al. 2022). Together with sbi-miR169, these miRNAs form an intricate regulatory network that supports sucrose accumulation in sugarcane.

13.7 Role of miRNAs in Abiotic Stress Tolerance in Sugarcane

In field conditions, plants are exposed to various abiotic stresses, either individually or in combination, which can significantly affect growth and yield (Zandalinas and Mittler 2022). These stresses seriously impact plant development, often resulting in oxidative damage to lipids, proteins, and DNA. They also cause the abnormal accumulation of molecules such as osmolytes (in cases of drought and salinity stress) or

reactive oxygen species (ROS) during oxidative stress. Given these challenges, there is a pressing need for innovative techniques that can help plant scientists create stress-resilient crops (Verma et al. 2021a, b, 2023b). Recent advancements in molecular biology have underscored the role of microRNAs (miRNAs) in crop improvement.

These small RNA molecules naturally modulate gene expression across various pathways, and environmental stresses can trigger changes in their expression (Verma et al. 2021c, d, 2023c, d). This unique response suggests that miRNAs could be valuable targets for enhancing stress tolerance in plants. The behavior of stress-responsive miRNAs varies depending on the miRNA, the type of stress, the plant genotype, and the modulation of miRNA–protein interactions. Additionally, miRNA activity may depend on the number of target genes involved (Swapna and Kumar 2017; Verma et al. 2022c, 2024c,d). Typically, stress-suppressed miRNAs enhance the accumulation of positive regulators for stress tolerance, while stress-induced miRNAs repress negative regulators. Recent findings of miRNA target genes related to stress tolerance in sugarcane underscore the potential of miRNAs in boosting plant resistance to a range of abiotic stresses (Meena et al. 2020).

13.7.1 Drought Stress

Drought is now one of the most critical abiotic stressors affecting crop production due to ongoing climate changes (Verma et al. 2021e, f). Researchers have used miRNA profiling better to understand the molecular mechanisms underlying drought tolerance in crops. Extensive studies have focused on the role of miRNAs in drought tolerance in sugarcane (Verma et al. 2020f). For example, Ferreira et al. (2012) reported that miRNA expression varies by cultivar under drought stress, with specific miRNAs being upregulated in one cultivar while downregulated in another. Studies indicate that sugarcane's drought response includes the up-regulation of miR393. A total of 18 miRNA families have been identified in sugarcane cultivars with varying drought tolerance, seven of which exhibit differential expression under drought, influencing detected levels.

RT-qPCR analysis confirmed the expression of five miRNAs (ssp-miR164, ssp-miR394, ssp-miR397, ssp-miR399-seq1, and miR528). Thiebaut et al. (2012a) used computational methods on four sugarcane genotypes exposed to stress, identifying 623 new mature miRNAs, 44 of which were high confidence. The water deficiency assay revealed 67 miRNAs, with 20 common across susceptible and tolerant assays. Predicted target analyses suggested miRNA targets include zinc-finger proteins and serine/threonine kinases. In the drought-tolerant cultivar ROC22, Lin et al. (2014) identified 438 target genes in leaves alongside 23 conserved and 34 novel miRNAs. Eleven miRNAs showed differential expression between control and drought conditions, with nine families up-regulated and two down-regulated (Verma et al. 2024a, b). Gentile et al. (2013) studied two sugarcane cultivars with differing drought tolerance and identified 18 miRNA families, with 30 mature miRNAs and 13

differentially expressed in both cultivars. These findings suggest that genetic background may influence miRNA expression under drought, enabling sugarcane to adapt to various drought stages and intensities.

13.7.2 Waterlogging

Sugarcane's tolerance or susceptibility to waterlogging is likely linked to miRNA regulation. A study identified seven candidate miRNAs in plants subjected to 1 month of waterlogging. Additionally, 26 conserved miRNA families, two novel miRNAs, and multiple trans-acting siRNAs were identified in sugarcane axillary buds using bioinformatics, suggesting a unique regulatory response in sugarcane (Khan et al. 2014).

13.7.3 Salinity Stress

Soil salinity presents a critical environmental challenge to global food security (Raza et al. 2022). MicroRNAs (miRNAs) have become crucial for understanding and enhancing crop tolerance to salt stress. Preliminary evidence from Patade and Suprasanna (2010) suggests that miR159 contributes to sugarcane's response to abiotic stress. Their study used computational tools to predict that MYB transcription factors could be targeted by miR159, with a noted increase in miR159 transcripts during brief salt stress exposure. A regulatory connection may be inferred from a slight downregulation of miR159 alongside MYB gene upregulation after an hour of salt stress. Similarly, Carnavale Bottino et al. (2013) identified 11 miRNAs with increased expression in severely salt-stressed Saccharum spp. cultivars compared to mildly stressed ones, highlighting miRNAs' importance in managing salinity tolerance, which appears to depend on stress type and duration.

13.7.4 Cold Stress

Extreme temperatures, including cold stress, heavily impact plant growth, development, and postharvest quality (Raza et al. 2021). Sugarcane is notably sensitive to cold, with miRNAs playing a key role in regulating stress response mechanisms. Thiebaut et al. (2012b) found upregulated miR319 in sugarcane plantlets exposed to 4 °C for 24 hours, noted in both roots and shoots. A related study by Yang YuTing et al. (2017) identified 412 sugarcane miRNAs, with 62 showing differential expression under cold stress, while Yang et al. (2018) detected 144 miRNAs responsive to cold using RNA sequencing, with 32 showing differential expression in cold-tolerant and sensitive sugarcane cultivars (FN39 and ROC22). To further explore

miRNA roles, ScmiR393 was overexpressed in *Arabidopsis,* enhancing cold tolerance by increasing anthocyanin and proline, reducing MDA and H2O2 levels, and boosting SOD, POD, and CAT activities. Transcriptome analysis showed that ScmiR393 hindered the auxin signaling pathway by targeting TIR1, reducing ethylene signaling and promoting cold-resistant gene expression. This work strengthens the understanding of ScmiR393's role in sugarcane's cold stress response.

13.7.5 Aluminum Stress Tolerance

Aluminum toxicity (Al3+) is a major agricultural limitation in acidic soils, releasing toxic Al3+ ions at low pH levels that impede root growth and affect plant oxidative and molecular metabolism. Silva et al. (2019) identified 394 differentially expressed miRNAs in two sugarcane cultivars with contrasting aluminum tolerance, finding 104 miRNAs unique to the tolerant cultivar, 116 to the sensitive, and 87 common to both. In the tolerant cultivar, 52% of miRNAs were upregulated, while most miRNAs in the sensitive cultivar were downregulated. In a follow-up study, Silva et al. (2021) identified two potential regulatory mechanisms in the tolerant genotype (CTC-2) under Al3+ stress, with miR395 involved in detoxification and miR160, miR6225-5p, and miR167 contributing to lateral root formation, enhancing the genotype's tolerance.

13.7.6 Low-Potassium Stress Tolerance

Potassium is essential for plant growth, as it regulates osmotic pressure, activates enzymes, and supports key physiological functions. However, its bioavailability in soil is limited, making potassium deficiency a significant threat to sustainable agriculture. Understanding genetic and regulatory systems that enable plant tolerance to low potassium can support the development of potassium-efficient crop varieties. Zhang et al. (2022) identified 324 known and 42 novel miRNAs in sugarcane roots under low-potassium conditions, with 36 showing differential expression. The study suggests that miR156-x/z and miR171-x may regulate ethylene signaling pathways and lateral root development.

13.7.7 Low-Nitrogen Stress Tolerance

Chemical nitrogen (N) fertilizers are widely used in sugarcane cultivation. However, understanding the molecular processes involved in nitrogen use efficiency (NUE) and identifying miRNAs that regulate these pathways in sugarcane is essential to breeding nitrogen-efficient varieties. Such improvements can ultimately reduce

fertilizer dependency. In a recent study by Gao et al. (2022), transcriptome data were analyzed, resulting in a root miRNA database for the nitrogen-efficient sugarcane variety ROC22 under 3 h of low-nitrogen stress. The primary aims were to identify miRNAs that could enhance sugarcane NUE and to unravel the regulatory network governing its low nitrogen response. The study identified 78 miRNAs across six libraries, with 15 showing differential expression in ROC22 roots under low nitrogen conditions. Notably, miR156 was significantly upregulated, while miR393 and miR396 were strongly downregulated. Among the novel miRNAs, seven (Novel-22, Novel-23, Novel-47, Novel-57, Novel-122, Novel-158, and Novel-169) were upregulated, while five (Novel-15, Novel-31, Novel-173, Novel-175, and Novel-215) were downregulated under stress.

These findings highlight the essential role of miRNAs in sugarcane's abiotic stress tolerance by modulating genes linked to stress detoxification, root development, and signal pathways. Expanding our knowledge and manipulating these miRNAs offers promising strategies for breeding stress-tolerant sugarcane cultivars (Table 13.1).

13.8 Role of miRNAs in Biotic Stress Tolerance in Sugarcane

MicroRNAs are crucial in the defense mechanisms of sugarcane against biotic stresses induced by pathogens, pests, and viruses. By regulating gene expression, these miRNAs modulate the activity of target genes involved in stress responses, enhancing the plant's resistance. Upon the invasion of pathogens, specific miRNAs show increased expression, which activates defense pathways and modifies hormone signaling, particularly within the salicylic acid (SA) and jasmonic acid (JA) signaling pathways (Nandakumar et al. 2021; Verma et al. 2022d, e). Various biotic stresses, including fungal infections, bacterial pathogens, viral attacks, and insect infestations, severely impact sugarcane yield. miRNAs play a significant role in mitigating these losses by influencing pathogen recognition and the expression of defense-related genes. In sugarcane infected by *Acidovorax avenae* ssp. *avenae,* the causative agent of red stripe disease, over 240 miRNAs have been identified, highlighting the importance of miRNAs in providing resistance against bacterial diseases (Thiebaut et al. 2012a).

Moreover, both miR408 and miR397 have been observed to be downregulated in plants infected by pathogens. miR408 also showed decreased levels in sugarcane infected with the fungal pathogen *Puccinia kuehnii,* responsible for orange rust. This miRNA regulates a laccase gene, and its expression profile is inversely correlated with its target, suggesting its critical role in sugarcane's interactions with microorganisms. Notably, the behavior of miR408 varies depending on the type of microbial interaction. Instead of suppression during pathogenic attacks, miR408 was significantly upregulated in sugarcane inoculated with the beneficial bacterium *Gluconacetobacter diazotrophicus* (Thiebaut et al. 2017; Verma et al. 2022f, g). Viral threats, such as the sugarcane mosaic virus (SCMV) and sugarcane

Table 13.1 List of miRNAs for abiotic stress tolerance in sugarcane crop

Abiotic stress	miRNAs responsive to stress along with target description	References
Drought	ssp-miR164—Targets NAC transcription factors and MDR-like ABC transporters ssp-miR393—Linked with Auxin-responsive factor TIR1-like protein ssp-miR394—Interacts with Glyceraldehyde 3-Phosphate Dehydrogenase and Protein N5P-interacting kinase 1-like ssp-miR397– Targets Laccase 23-like proteins ssp-miR399—Involved in the regulation of Inorganic pyrophosphatase 2-like proteins and Senescence-associated proteins ssp-miR528—Impacts UBX Domain Containing Proteins and Pyruvate dehydrogenase E1 alpha subunit ssp-miR1432—Regulates B-ZIP transcription factors, including ABRE-binding factor BZ-1bZIP sspmiR160-seq1—Targets NAC domain-containing protein 68-like sspmiR166-seq3—Associated with Homeobox-leucine zipper protein HOX32 sspmiR169-seq2– Controls Nuclear transcription factor Y subunit A-10 sspmiR171-seq2– Linked with Scl1 protein sspmiR172—Targets Floral homeotic protein APETALA 2-like sof-miR156—Squamosa promoter binding proteins sof-miR159—MYB proteins sof-miR167—Regulates Auxin response factor sof-miR168– Targets AGO1-like protein sof-miR396—Involved with Nucleic acid binding proteins sof-miR408—Interacts with blue copper proteins miR-156—Associated with Squamosa promoter binding proteins miR-166—G-type lectin S receptor-like serine/threonine protein kinase miR-444a—Putative RING/U-box superfamily protein, CTL-like protein DDB 1miR-444b—No identified homology and sugar transporter activity miR-5564—Related to Rf-1 gene, a fertility restorer	Ferreira et al. (2012) Gentile et al. (2015) Lin et al. (2014)
Salinity	miR159– Targets MYB proteins and GAMyb proteins miR169—Associated with HAP12 CCAAT-box transcription factors miR156 V– Regulates Squamosa Promoter Binding Domain Protein 5 miR159 XVI—Influences GAMyb protein activity miR166 III—Controls Class III HD-Zip protein 4 miR167 V—Targets Auxin Response Factor 17 miR168 II—Linked with Argonaute 1 protein miR169 III—Engages HAP12 in the CCAAT-box transcription factor complex miR396 II—Affects Growth-Regulating Factor 1 miR397 III—Acts on Laccase-10 precursor protein miR398—Involved with Selenium-binding protein miR398—Interacts with Serine/Threonine Kinase-like protein miR528 I—Associated with Putative Laccase activity.	Carnavale Bottino et al. (2013)

(continued)

Table 13.1 (continued)

Abiotic stress	miRNAs responsive to stress along with target description	References
Cold	miR319, miR5059, miR5072, miR5077, and miR8155, miR5152-3p, miR5813 miR2199 and miR5054	Thiebaut et al. (2012b); Yang YuTing et al. (2017)
Aluminium toxicity	miR121—Associated with K+ Uptake Permease miR156—Targets Squamosa Promoter-Binding Protein-like miR159– Engages MYB Domain Protein and LRR Protein miR160—Involved with Auxin Response Factor miR164–Regulates NAC Domain Containing Protein miR167–Linked to OsWAK and Copper-Transporting ATPase PAA1 miR169—Acts on 12-Oxo-Phytodienoic Acid Reductase miR319– Influences MYB Domain Protein miR396– Controls Growth-Regulating Factor miR398–Targets Copper/Zinc Superoxide Dismutase miR444–Associated with MADS-Box Transcription Factor	Silva et al. (2019)
	miR395– Involved in sulfate transmembrane transporter activity and sulfate adenylyltransferase activity miR6225-5p—Associated with ATPase activity, microtubule binding, and protein phosphate regulator activity miR160– Engages in DNA-binding transcription factor activity miR159—Functions in aminocyclopropane-1-carboxylate synthase activity, protein binding, and DNA-binding transcription factor activity miR167—Linked to DNA-binding and protein-binding activities miR168– Plays a role in allantoate deiminase activity, metallopeptidase activity, endoribonuclease activity, and mRNA and protein binding	Silva et al. (2021)
Low Potassium	miR397-x, miR156-z/x, and miR5054-z, miR7741-y and miR1848-z, miR7720-x, miR528-x	Zhang et al. (2022)
Low Nitrogen	miR396 (down-regulated) miR393 (down-regulated) and miR156 (up-regulated)	Gao et al. (2022)

bacilliform virus (SCBV), pose severe risks to sugarcane crops (Sawitri et al. 2024; Ashraf et al. 2022). miRNAs protect against viral infections by downregulating viral gene expression (Wu et al. 2015). The research focused on SCMV, which is recognized as the most damaging viral agent to sugarcane, and utilized in silico methods to identify miRNAs that target SCMV-positive sense, single-stranded RNA-encoded mRNAs (Table 13.2).

This strategy initiates the RNA interference (RNAi) pathway, presenting a significant approach for developing SCMV-resistant sugarcane cultivars. Recent investigations have profiled 1578 miRNAs from 30 small RNA libraries, encompassing 660 known and 380 novel miRNAs. Several miRNA homologs, such as sof-miR159c

Table 13.2 Summarizing key miRNAs in sugarcane associated with biotic stress

miRNA	Associated trait/ pathogen	Target strategy	Regulation mechanism	References
miR160	Sugarcane Mosaic Virus (SCMV)	CAB2R protein genes associated with chloroplast and light-harvesting complex.	Upregulation of miRNA	Yuan et al. (2024)
sof-miR159c		Cylindrical inclusion (CI) helicase ORF of SCMV	Down-regulation of Target	Wenzhi et al. (2024)
sof-miR396	Sugarcane Bacilliform Guadeloupe A Virus (SCBGAV)	ORF3 of the SCBGAV genome	Down-regulation of Target	Ashraf et al. (2020)
sof-miR159e, ssp-miR444	Sugarcane bacilliform virus (SCBV)	ORF3 of SCBV genome	Down-regulation of Target	Ashraf et al. (2022)
ssp-miR528	Sugarcane Yellow Leaf Virus (SCYLV)	ORF5 of the SCYLV genome	Down-regulation of Target	Ashraf et al. (2021)
miR444b.2	*Colletotrichum falcatum*	Genes involved in basal defense and signaling	Upregulation of miRNA	Nandakumar et al. (2021)
miR444a		Genes play a role in fundamental metabolic processes, transmembrane signaling, and the synthesis of amino acids.	Upregulation of miRNA	
miR5568b		Genes that contribute to the functions of chloroplasts and mitochondria promote the accumulation of jasmonic acid (JA) and salicylic acid (SA).	Upregulation of miRNA	
miR169b.3p		Genes that are engaged in the biosynthesis of phenylpropanoids and the hypersensitive response (HR)	Upregulation of miRNA	
miR166b.5p		Activation of SA pathway, calcium signaling	Upregulation of miRNA	

(continued)

Table 13.2 (continued)

miRNA	Associated trait/ pathogen	Target strategy	Regulation mechanism	References
miR858b	*Sporisorium scitamineum*	Myb-related protein Hv33 (MYB2)	Upregulation of miRNA	Su et al. (2019) Su et al. (2017)
miR168a-5p		Protein argonaute 1B (AGO 1B)	Downregulation of miRNA	
miR5783		PKA (Protein Kinase A)	Upregulation of miRNA	
miR5221		Auxin transporter, HSP72 (AUX1), Heat Shock Protein 72 (HSP 72)	Downregulation of miRNA	
miR6478		Constitutive Triple Response 1 (CTR1)	Downregulation of miRNA	
miR5054		PEX5 (Peroxisome 5)	Downregulation of miRNA	

(Wenzhi et al. 2024), along with miR171 and miR160 (Yuan et al. 2024), exhibit specific expression patterns associated with disease-resistance genes in SCMV-infected sugarcane. Among these, the miR160 family, initially characterized in Arabidopsis as a regulator of auxin-response factors (Rhoades et al. 2002), plays diverse roles in sugarcane disease resistance. The upregulation of miR160 enhances the inhibition of the CABR2 gene, which could potentially weaken chloroplast defenses (Yuan et al. 2024). Furthermore, sof-miR159c has been identified as a high-confidence target site for the Potyvirus cylindrical inclusion helicase (CI) open reading frame (Wenzhi et al. 2024). Further, in silico analysis identified 14 miRNAs that target SCBV genome components, with sof-miR159 and sof-miR396 highlighted as key miRNAs against SCBV (Ashraf et al. 2022).

One significant fungal disease impacting sugarcane is red rot, caused by *Colletotrichum falcatum*. Several miRNAs, including miR444, miR5568, miR169, miR166, miR164, and miR162, have been recognized as active participants in the plant's response to fungal stress. For instance, miR444b.2 regulates genes involved in basal defense and signaling, while miR5568b supports chloroplast and mitochondrial functions, enhances the hypersensitive response (HR), and promotes the accumulation of SA and JA. Similarly, the fungal-responsive miRNA miR169b.3p regulates phenylpropanoid biosynthesis, post-transcriptional gene regulation, and transport activity to aid the HR response. Other defense-related miRNAs include miR166b.5p, which contributes to resistance enhancement, miR164c, involved in proline metabolism, and miR162b, which plays a role in miRNA biogenesis and chromatin modification (Nandakumar et al. 2021).

During interactions between sugarcane and *Sporisorium scitamineum,* the agent responsible for smut disease, miR5671, miR5054, miR5783, miR5221, and miR6478 has been shown to target genes involved in the mitogen-activated protein kinase (MAPK) signaling pathway, plant hormone signal transduction, and plant–pathogen interactions (Su et al. 2017). Degradome sequencing effectively identifies

miRNAs and their target genes during plant development or under stress conditions (Lin et al. 2019). This method has been utilized to map miRNA-target interactions in sugarcane under biotic stress. In the case of *Sporisorium scitamineum* infection, 97 known and 112 novel miRNAs, along with 309 predicted target genes, were identified, underscoring the regulatory dynamics between miRNAs and their targets (Su et al. 2019). These findings reveal the intricate regulatory network governed by miRNAs in sugarcane during biotic stress, which can be leveraged for developing resistant varieties through biotechnological methods.

13.9 Future Prospects and Challenges

The future of miRNA-based techniques for sugarcane improvement is promising yet challenged by significant obstacles. miRNAs are established as critical regulators of developmental timing, flowering, and stress responses in crops, such as *Arabidopsis* (Aukerman and Sakai 2003), and their roles in sugarcane development are gradually being revealed. In sugarcane, miRNAs influence genotype transitions, impacting maturity timing and sugar accumulation, which differ across early- and late-maturing varieties. By manipulating miRNA expression at key developmental stages, it may be possible to enhance sucrose accumulation (Swapna and Kumar 2017). An exciting prospect in sugarcane research is harnessing miRNA regulation to fine-tune metabolic processes that directly impact essential traits, including yield, biomass, and stress tolerance (Mandal et al. 2021). miRNAs offer substantial potential in sugarcane improvement by regulating key traits such as biomass yield and stress tolerance. Targeting miRNAs such as miR156 and miR169 could greatly enhance biomass and sugar accumulation, supporting a more sustainable biofuel industry. Nonetheless, the intricate nature of miRNA networks calls for a cautious and precise approach to genetic manipulation. Future research integrating omics and advanced breeding techniques will be essential to overcome current limitations and develop robust, high-yield, and resilient sugarcane varieties to unlock the full potential of miRNA-mediated regulation in sugarcane.

miRNAs ability to target multiple genes offers a sophisticated approach to improving these traits through multigene control. For instance, artificial miRNAs (amiRNAs) have been suggested as tools to selectively knock down specific gene functions. However, to prevent unintentional gene suppression or ineffective regulation, optimizing amiRNA expression remains crucial. One significant advantage of these techniques, especially with amiRNAs and CRISPR/Cas systems, is their precision; they achieve high specificity and minimize off-target effects. Techniques such as topical delivery and epigenetic memory manipulation could avoid permanent genetic modification, enabling crops to remain non-GMO, reducing regulatory hurdles, and improving public acceptance. CRISPR and epigenetic modifications offer the potential for heritable traits, thereby expanding breeding applications for stress-resistant crops. Nonetheless, several challenges exist. Potential challenges include regulatory hurdles and public concern over genetically modified organisms

(GMOs), especially for food crops. There are also risks of pleiotropic effects, where unintended traits appear due to overexpression or suppression of miRNAs. Techniques such as amiRNAs and target mimicry are technically demanding, requiring significant bioinformatics expertise and a deep understanding of gene interactions, which can limit accessibility.

Designing amiRNAs requires extensive knowledge of target gene sequences, their interactions, and bioinformatics support to ensure accuracy. Moreover, expressing amiRNAs in plants can sometimes be inefficient due to incomplete matching with target sequences. In the case of target mimicry establishing an optimal mimic, the sequence is complex and can be limited by the stability of the mimic in different plant environments. Editing can sometimes yield unintended mutations or off-target effects. Public and regulatory concerns also pose challenges; transgenesis and CRISPR-based techniques often face skepticism and strict regulations, especially in regions with stringent GMO laws. Achieving stable miRNA levels and uniform uptake across various tissues can be challenging with topical miRNA delivery, often requiring repeated applications for prolonged effects, which may demand substantial resources. Similarly, precise control over methylation extent and targeting is difficult with epigenetic memory, with potential off-target effects complicating intended results. Moreover, predicting the durability of epigenetic modifications across diverse environments adds a layer of complexity.

RT-qPCR requires careful optimization of primers for miRNA specificity, especially for closely related miRNAs. Overexpression methodologies may lead to off-target effects, as miRNAs can sometimes interact with unintended targets. Creating stable transgenic lines can also be resource-intensive and subject to regulatory hurdles. Alternative strategies are under exploration to overcome these hurdles. For instance, incorporating stress-inducible promoters could allow more controlled expression of MIR genes, reducing the adverse effects linked with constant expression. Using viral vectors for temporary miRNA overexpression or knockdown represents another promising alternative, enabling functional studies without permanent genomic changes. These advanced techniques provide innovative means to optimize plant stress responses, balancing efficiency and specificity against challenges such as stability, unintended effects, and regulatory acceptance.

13.10 Conclusions

Despite the promising potential of miRNA technologies, several challenges remain. Sugarcane's high ploidy level and intricate regulatory networks complicate miRNA-mediated gene regulation. Understanding miRNA interactions with other regulatory mechanisms, such as transcriptional and post-transcriptional controls, is necessary to pinpoint how miRNAs influence traits such as sucrose accumulation. Complicating factors include duplicate genes in sugarcane, more likely targets of miRNAs than single genes (Wang and Adams 2015), raising further questions about the influence of ploidy and genome complexity on miRNA activity. Besides, translating

miRNA-based genetic techniques from the lab to the field is technically and regulatory complex. Constructs regulating miRNA expression may fall under GMO regulations. Although miRNA-based modifications focus on non-coding genes rather than protein-coding regions, they still encounter scrutiny, especially concerning off-target effects and potential environmental impacts (Mandal et al. 2021). The possibility of miRNAs unintentionally targeting non-specific genes could result in unforeseen changes to plant development, growth, or resistance. The potential for cross-kingdom miRNA transfer, for instance, through food or soil microbes, further complicates safety assessments. Comparative studies, in-depth risk assessments, and long-term field trials will ensure the safe application of miRNA technologies in sugarcane and other crops.

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Chapter 14
Genomics and Prospects for Molecular Breeding in Sugarcane

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Abstract Given its high yield and extensive cultivation, the rising global demand for sustainable energy sources has made sugarcane a key candidate for biofuel production. However, the genetic improvement of sugarcane through conventional breeding has reached a plateau, largely due to prolonged breeding cycles and limited genetic diversity. Emerging technologies, such as long-read sequencing have improved genome assembly accuracy; yet integrating this genomic data into practical breeding programs remains a significant hurdle. Ongoing global research and collaboration are crucial for leveraging sugarcane's genetic potential to enhance its productivity and resilience for biofuel production. Recent advancements, including the "Sugarcane 100K SNP Array," have enabled high-resolution mapping of quantitative trait loci (QTLs), critical for improving yield, disease resistance, and stress tolerance. In addition to mapping, functional genomics techniques, such as RNA interference (RNAi) and CRISPR-Cas9 gene editing have been pivotal in dissecting gene functions. Molecular breeding approaches, including marker-assisted selection (MAS), genomic selection (GS), quantitative trait loci (QTL) mapping, and CRISPR-Cas9 gene editing, have significantly advanced sugarcane breeding for sustainable agriculture.

Keywords Sugarcane · Breeding targets · Genomics · Stress · QTLs and genes · Genetic maps · Genome editing · SNP array

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14.1 Introduction

With the rise of global warming, one of the major concerns has become finding alternatives for biofuel, for which sugarcane is a promising crop for bioenergy and biofuel production, as it is also one of the most harvested crops by tonnage (Oliveira et al. 2022; Verma et al. 2024a, b). Though conventional breeding produced sugarcane varieties with increased sugar output that can withstand biotic and abiotic stresses, the performance of the varieties has plateaued (Liang et al. 2024). The productivity of these varieties may have been created due to longer breeding cycles and limited genetic diversity among breeding populations because the beginning clones of today's commercial sugarcane hybrids were created by crossing two foundation species (*Saccharum officinarum* and *Saccharum spontaneum*). Sugarcane is a monocotyledon and belongs to the genus *Saccharum* of the tribe Andropogoneae, sub-tribe Saccharineae in the family Poaceae (Afghan et al. 2024).

Depending on taxonomic interpretation, the genus *Saccharum* consists of 6–37 species. The members are of tall grasses, native to warm temperate to tropical regions of South and South East Asia. Sugarcane has thick, jointed and fibrous stalks 2–6 meters tall that store sugar. There are four cultivated species, namely *S. officinarum* (noble cane, $2n = 80$), *S. barberi* ($2n = 82–124$), *S. sinense* ($2n = 111–120$), and two wild species, namely *S. spontaneum* ($2n = 40–128$) and *S. robustum* ($2n = 60–194$) (Gupta et al. 2010). *Saccharum* species complex has been widely cultivated in India since its nobilization because of its high sucrose content and adaptability to adverse environmental conditions. Sugarcane contributes approximately 80% of the global sugar requirement; sugar beet meets the remainder (Verma et al. 2020a; Zheng et al. 2023; Chen et al. 2024). Brazil, India, Thailand, China, the USA, Mexico, Pakistan, Australia and Guatemala are the major sugarcane-producing countries (http://www.fao.org/faostat/en).

14.2 Sugarcane Genome: Complexity and Structure

14.2.1 Genome Complexity

With additional backcrossing with *S. officinarum*, modern sugarcane hybrids originated from interspecific crosses between the wild, thin-stalked, low-sugar *S. spontaneum* and the thick-stalked, high-sugar *S. officinarum*. In addition to improving the present farmed sugarcane hybrids' vigor, robustness, tillering, disease resistance, and environmental adaptability, this complicated hybridization process increased the genome's complexity beyond that of their ancestors (Verma et al. 2020b, 2021a, b). A mixture of aneuploid and homo(eo)logous chromosomes that were unevenly inherited from the two polyploid progenitor species makes up the resulting hybrid genome, which has a massive size of about 10 Gb. Hybrids might have between 100 and 130 chromosomes, depending on the particular cross.

Depending on the specific cross, hybrids might have between 100 and 130 chromosomes. Around 70–80% of these chromosomes come from *S. officinarum*, 10–20% from *S. spontaneum*, and 10% are the product of interspecific recombination (Bao et al. 2024; Verma et al. 2023a, b, 2024c).

14.2.2 Genome Sequencing Efforts

S. officinarum and *S. spontaneum* are the leading representatives of sugarcane, which has seen extensive genome sequencing research to understand its complicated polyploidy better and improve breeding techniques. By successfully creating a reference genome for *S. spontaneum*, the Sugarcane Genome Sequencing Consortium (SGSC) demonstrated the plant's varied genetic composition and revealed a sizable genomic structure with roughly 10 chromosomes. Prominent initiatives, including the Chinese Academy of Agricultural Sciences, have concentrated on sequencing several *S. officinarum* cultivars and determining significant sugar yield and stress resistance characteristics (Verma et al. 2020c, 2022a, b). However, the high heterozygosity and polyploid nature of the genome pose difficulties for these efforts, making the procedures of assembly and annotation more difficult. Advanced sequencing technologies such as long-read sequencing have been used to overcome these obstacles and enable more precise genome assembly (Singh et al. 2020; Guo et al. 2021, 2022). Despite these developments, there is still a barrier to incorporating genomic data into applicable breeding plans, which calls for continued study and cooperation on a global scale (Singh et al. 2019; Verma et al. 2020d, e; Wu et al. 2022; Jagadesh et al. 2024). Unlocking sugarcane's genetic potential and enhancing its resilience and production in agricultural settings depend heavily on these extensive sequencing efforts (Zhang et al. 2018; Sharma et al. 2021).

14.2.3 Recent Advances in Genome Sequencing

The field of sugarcane genomics has seen substantial change due to recent developments in genome sequencing technologies, especially long-read sequencing and high-throughput methods. Researchers can now produce contiguous sequences that cover enormous repetitive areas and intricate structural changes in the sugarcane genome thanks to long-read sequencing techniques like those created by Oxford Nanopore Technologies and Pacific Biosciences. For both *S. officinarum* and *S. spontaneum*, this ability has resulted in more precise and comprehensive genome assemblies, improving the annotation of genes linked to critical characteristics such as disease resistance and sugar yield (Song et al. 2019; Chen et al. 2020; Huang et al. 2022). Furthermore, high-throughput sequencing platforms, such as the most recent versions of Illumina technology, allow for the quick and economical sequencing of several sugarcane cultivars, opening the door to in-depth genomic analysis

and genome-wide association studies (GWAS). These developments have not only improved our knowledge of the genetic diversity of sugarcane but have also sped up the search for genomic markers that may be applied to breeding initiatives that aim to increase crop yield and resilience (Verma et al. 2019a, b, 2020f; Kumari et al. 2023; Huang et al. 2024). Unlocking sugarcane's genetic potential and opening the door to more sustainable farming methods depend on the integration of these cutting-edge sequencing technologies (Solanki et al. 2023; Chen et al. 2023a).

14.2.4 Exploration of Targets for Breeding

In hybrid breeding projects, many important features for sugarcane improvement depend on dosage and are polymorphic in the progenitor species. While high sugar content necessitates additive contributions of gene copies from *S. officinarum*, brown rust resistance seems to be derived from a single-copy location within the genome. During the primary assembly and annotation, we recorded copy number and protein sequence variation between and within R570 progenitor sub-genomes to expedite comparable breeding operations and provide marker-assisted selection strategies.

14.3 Genomic Resources for Sugarcane Breeding

14.3.1 Marker Density and Genetic Map

In sugarcane, molecular markers, specifically simple sequence repeats (SSRs) and single nucleotide polymorphisms (SNPs) have been essential in creating high-density genetic maps. Traditional breeding and mapping techniques are ineffective because of sugarcane's complicated polyploidy, high degree of heterozygosity, and huge genome size. By creating and implementing these markers, researchers can overcome these obstacles. SSRs and SNPs in sugarcane genomics have advanced significantly since 2020, facilitating marker-assisted selection (MAS) and speeding up genetic advancements. The genome contains tandemly repeated DNA sequences called microsatellites, or SSRs. SSRs are helpful for distinguishing closely related genotypes due to their co-dominance and high polymorphism rates (Verma et al. 2021c, d; Anas et al. 2021a, b). Although developing SSR markers has historically required a lot of resources, current developments in next-generation sequencing (NGS) technologies have made SSR identification easier and more accessible for sugarcane study (Verma et al. 2021e; Liang et al. 2024). In sugarcane, SSR markers are crucial for generating genetic linkage maps, particularly for locating quantitative trait loci (QTLs) connected to characteristics like yield, disease resistance, and sucrose content.

Despite their shortcomings in high-throughput genotyping, SSRs are essential in sugarcane breeding efforts, especially in low-resource environments where whole-genome resequencing could be too expensive (Malviya et al. 2020; He et al. 2021). The most prevalent kind of molecular marker is SNPs, or single base-pair variations in the DNA sequence, which provide a more thorough and high-resolution method for genetic mapping. High-density genetic maps, which enable improved resolution and precision in mapping QTLs linked to economically significant traits, have been made possible by the enormous number of SNPs in the sugarcane genome. SNP arrays, such as the recently created "Sugarcane 100K SNP Array," enable high-throughput and economical genotyping, facilitating population-wide research and providing more understanding of complex features. This has been especially helpful in locating QTLs linked to biomass yield and abiotic stress tolerance (Verma et al. 2021f, 2022c, d), where high-resolution mapping is essential.

14.3.2 Construction of High-Density Genetic Maps

By combining SSRs and SNPs, scientists have been able to create more thorough genetic maps of sugarcane, identifying the chromosomal positions of genes that affect important agronomic aspects. SSR markers add robustness and dependability, particularly in polyploid mapping, while SNP markers offer higher density and resolution because of their abundance and genome-wide distribution. When combined, they have increased sugarcane genetic maps' precision and resolution, which is crucial for analyzing complex features influenced by several genes (Singh et al. 2021; Wu et al. 2022; Xu et al. 2024). Recent research has shown that it is possible to map highly duplicated regions and reveal the polyploid structure in sugarcane using high-density SNP markers, which will help with genomic selection tactics and advance trait selection for breeding. SSR and SNP markers have accelerated the creation of high-density genetic maps in sugarcane, allowing for more accurate QTL mapping and effective breeding for complex characteristics (Singh et al. 2021a, b). Sugarcane genomics is still developing with the introduction of new sequencing methods and SNP arrays, enabling quick and affordable advancements in this key crop (Xu et al. 2022).

14.3.3 Functional Genomics

Functional genomics uses RNA interference (RNAi), CRISPR-Cas systems, and gene knockouts to analyze how particular genes control key agronomic characteristics in sugarcane, including yield, stress tolerance, and disease resistance. By mutating or changing gene function, these technologies aid in the connection between genotype and phenotype and enable researchers to find and validate genes that are essential for enhancing agricultural performance. With the help of these

technologies, functional genomics in sugarcane, a genetically complicated polyploid, has advanced, providing accurate methods for identifying and examining gene functions even inside its highly repetitive genome.

14.3.4 Gene Knockouts and CRISPR-Cas9 Gene Editing

Gene knockout techniques are used to permanently block genes and examine the phenotypic alterations that ensue, frequently via conventional mutagenesis or CRISPR-based tactics. Specific sucrose transporter gene knockouts in sugarcane have shown their functions in sugar transport and accumulation pathways, directly impacting yield (Malviya et al. 2022; Guo et al. 2023; Li et al. 2023). By demonstrating a lower sucrose content in the knockout plants, for instance, knockout studies of the sucrose transporter SUT1 gene have confirmed its significance in the sugar synthesis pathway and validated its function in sugar partitioning and high-yield potential (Almeida et al. 2021; Verma et al. 2022e, 2023c). Genes linked to lignin biosynthesis have also been studied using gene knockout techniques. When these genes are decreased, biomass conversion efficiency in biofuel production can be increased, adding more economic value to sugarcane crops (De Moraes et al. 2022; Verma et al. 2024d, e).

As it allows for precise and targeted gene editing, the CRISPR-Cas9 system has emerged as a groundbreaking tool in functional genomics. CRISPR has been utilized to create sugarcane plants with increased disease resistance by focusing on genes linked to susceptibility. For example, the ShMYB gene linked to rust disease susceptibility was knocked out using CRISPR-Cas9, producing more resilient plants to this economically devastating disease (Sharma et al. 2022; Krishna et al. 2022; Malviya et al. 2024). Similarly, scientists have employed CRISPR to modify salt and drought tolerance genes by focusing on regulatory genes in the abscisic acid (ABA) pathway. This has resulted in sugarcane cultivars that are more resilient to stress in saline and water-limited environments (Verma et al. 2022e). Because of its accuracy and reproducibility, CRISPR is especially useful in sugarcane, a crop whose complicated genome and lengthy breeding cycles make conventional breeding difficult.

14.3.5 RNA Interference (RNAi)

RNA interference (RNAi) technology makes reversible post-transcriptional gene silencing possible, which permits functional genomics research without requiring irreversible gene disruption. The research of genes influencing yield-related variables in sugarcane has significantly benefited from using RNA interference. For instance, it is still necessary to analyze how the enzyme activities engaged in sucrose metabolism, the regulatory mechanisms for source-sink communication, and the

function of post-transcriptional control of sucrose-induced regulation of translation (SIRT) interact and to study the outcome of RNAi-mediated silencing of the concerning genes, which is implicated in sucrose accumulation; this directly connects the gene to yield attributes (Mehdi et al. 2024). Plants with increased resistance to fungal infections like smut disease, a frequent hazard to sugarcane productivity, have been produced by using RNA interference (RNAi) to target genes linked to biotic stress responses, such as PR5, a pathogenesis-related protein gene. To increase a plant's resistance to abiotic conditions, including heat and drought, RNA interference has also been used to downregulate genes involved in oxidative stress pathways (Verma et al. 2022f, g; Kumar et al. 2024).

14.4 Molecular Breeding Approaches in Sugarcane

Molecular breeding approaches have significantly accelerated advancements in sugarcane, particularly through marker-assisted selection (MAS), genomic selection (GS), quantitative trait loci (QTL) mapping, and CRISPR gene-editing applications.

14.4.1 Marker-Assisted Selection (MAS)

MAS is a crucial technique for locating and introducing desirable traits, such as high yield and disease resistance, by utilizing molecular markers linked to these traits. Compared to traditional phenotypic selection, MAS has helped select superior sugarcane cultivars more effectively by focusing on disease resistance markers for features like ratoon stunting disease and brown rust resistance. Trait localization has been further made possible by the advent of single nucleotide polymorphism (SNP) microarrays, which have aided in the discovery of yield and disease-resistance markers (Aitken 2022). Breeders can find particular SNPs associated with these important genes and choose for those qualities by using marker-assisted selection (MAS). For instance, MAS has employed markers associated with ScSPS and ScCKX to select genotypes with high yield and sugar content.

14.4.2 Genomic Selection (GS)

GS improves MAS by predicting breeding values using genome-wide markers and enabling quicker genetic gains. This method has been beneficial in sugarcane breeding, where polyploidy and genetic complexity pose difficulties. Breeders can more precisely forecast and choose high-yield and disease-resistant genotypes thanks to the improved GS accuracy provided by high-throughput genotyping and machine learning. Research shows that GS effectively improves sugarcane, making it a

crucial component of contemporary breeding initiatives (Sandhu et al. 2022). Genomic Selection is superior in managing the intricate polyploid genome of sugarcane because it takes a step further by taking into account polygenic characteristics and genome-wide markers. In sugarcane, GS has been used to select for multi-trait improvement, especially to increase yield and sucrose content simultaneously (Verma et al. 2023d). Breeders can choose high-sugar-content genotypes early in the breeding cycle by utilizing genomic selection (GS) with SNP markers linked to these genes. This reduces the time it takes to see gains.

Breeders can use genomic selection (GS) and marker-assisted selection (MAS) more successfully and create novel cultivars more quickly, thanks to high-density genetic maps made with SSRs and SNPs. With the help of these molecular markers, traits that have historically been difficult to breed for because of complicated inheritance patterns, such as drought tolerance, disease resistance, and sugar production, can now be mapped and selected more effectively. By supporting more focused advancements, the use of MAS and GS in breeding programs guarantees that new sugarcane varieties satisfy the requirements of sustainable agriculture (He et al. 2021).

14.4.3 Quantitative Trait Loci (QTL)

Mapping QTL mapping finds the genetic loci linked to complex qualities that are usually regulated by several genes, such as biomass output, drought tolerance, and sugar content. The selection of elite varieties in sugarcane has been supported by QTL mapping, making identifying areas associated with yield and disease resistance easier. For example, QTLs linked to tillering ability and sucrose content have been discovered, laying the groundwork for upcoming breeding plans (Meena et al. 2022).

14.4.4 CRISPR-Cas9 Gene Editing

CRISPR technology has completely changed sugarcane breeding because it allows for precise gene alterations. CRISPR has been effectively employed by researchers to modify genes associated with drought tolerance and lignin biosynthesis, which are critical characteristics for the production of biofuel and climate resilience. By increasing yield potential under stress conditions and streamlining downstream processing for the generation of bioethanol, these improvements raise the agronomic value of sugarcane. Together, these molecular breeding techniques allow for the accurate selection and enhancement of desired sugarcane traits, overcoming obstacles brought on by the plant's complex genome and environmental stressors while promoting yield and sustainability increases (Aitken 2022; Meena et al. 2022; Xu et al. 2022).

14.5 Genomics-Assisted Breeding for Specific Traits

Genomics-assisted breeding in sugarcane aims to increase yield and sugar content using genome-wide association studies (GWAS), candidate gene identification, and genetic markers. This method speeds up the breeding cycle and improves results for qualities controlled by intricate genetic systems by enabling breeders to select for specific genes linked to desired traits. Here is a summary of the main genes related to sugar content and yield, along with how genomics-assisted breeding uses them:

14.5.1 Increasing Yield

Genes related to cell division, biomass generation, and general plant architecture significantly impact sugarcane yield. Breeding using genomics has revealed several important genes: Gibberellin-insensitive genes ScGAI and ScRGA are known to influence the regulation of plant development. Under the right circumstances, knocking down or altering these genes may increase yield by influencing biomass and stalk elongation. Cytokinin oxidase or ScCKX: Cytokinins are necessary for shoot growth and cell division. Controlling the expression of ScCKX genes can improve tillering and biomass buildup on the stalks. These genes are involved in the cytokinin degradation pathway. Sugar transporter genes are members of the ScSWEET family. By altering specific ScSWEET gene family members, sucrose mobilization is improved, perhaps improving carbon partitioning and increasing biomass in the stalk to increase yield. These genes are targeted by MAS and CRISPR-Cas gene editing to maximize growth-related characteristics, which in turn affect yield.

14.5.2 Enhancing Sugar Content

Genes governing sucrose accumulation and metabolic activity are the main determinants of sugarcane's desirable high-sugar content. Sucrose-phosphate synthase, or ScSPS: Sucrose synthesis depends on this enzyme. Higher sucrose buildup in the stalk is correlated with increased ScSPS expression. Breeders can increase the amount of sucrose in sugarcane tissues by encouraging the expression of this gene (Singh et al. 2023). The sucrose transporter 1(ScSUT1): The sucrose transporter protein that this gene encodes is in charge of sucrose loading into phloem tissues, facilitating the effective transfer of sugars to the stalk's storage tissues, and better sucrose buildup by breeding for increased ScSUT1 expression or efficiency. ScINV governs invertase; invertases control how sucrose is broken down into less complex sugars. Breeders can increase the amount of sugar in the stalk by decreasing the breakdown of sucrose by downregulating specific invertase isoforms, especially cell

wall invertase. The gene ScBADH (betaine aldehyde dehydrogenase) is linked to the manufacture of osmoprotectants. Higher tolerance to salt stress has been associated with increased expression of ScBADH, indirectly promoting sugar storage under stress by lessening osmotic stress on plant cells (Deng et al. 2023; Joshi et al. 2023; Bhatt et al. 2024).

14.6 Application of Genomics-Assisted Breeding Approaches

14.6.1 Marker-Assisted Selection (MAS)

Breeders can find particular SNPs associated with these important genes and choose those qualities using marker-assisted selection (MAS). For instance, MAS has employed markers associated with ScSPS and ScCKX to select genotypes with high yield and sugar content. Genomic Selection is superior in managing the intricate polyploid genome of sugarcane because it takes a step further by taking into account polygenic characteristics and genome-wide markers. In sugarcane, GS has been used to select for multi-trait improvement, significantly increasing yield and sucrose content simultaneously. Breeders can choose high-sugar-content genotypes early in the breeding cycle by utilizing genomic selection (GS) with SNP markers linked to these genes. This reduces the time it takes to see gains.

14.6.2 CRISPR-Cas9 Editing

Genomics-assisted techniques are revolutionizing sugarcane breeding by enabling precise, targeted improvements that speed up the development of high-yield, high-sucrose cultivars better suited to meet the demands of both the sugar and biofuel industries. CRISPR has been used to alter sugar transporter and growth regulator genes, directly contributing to higher sucrose levels and greater stalk biomass without negatively affecting plant health. This tool allows precise edits to genes like ScSUT1 and ScGAI to enhance specific traits.

14.6.3 Disease Resistance Using Resistant Markers

Genomics-assisted breeding has improved the control of important sugarcane diseases by developing markers, identifying genes, and using resistance markers. A summary of genomics-enabled resistance breeding techniques and pertinent genes linked to the main sugarcane diseases is provided below:

14.6.3.1 Red Rot

Red rot, a severe sugarcane disease brought on by *Colletotrichum falcatum*, severely reduces yields and the quality of sucrose by decomposing the stalk tissues. Significant quantitative trait loci (QTLs) associated with resistance to red rot have been found using genome-wide association studies (GWAS). For example, resistance was associated with a large QTL region containing a chitinase A gene cluster. Chitinases are known to improve defense by breaking down fungal cell walls. Red rot resistance is a required characteristic in new sugarcane varieties in India, and markers for the genes in this QTL have been designed to help with selection precision. The genetic basis of red rot resistance and the potential for QTL-based marker-assisted selection (MAS) to improve breeding efforts aimed at this disease, especially in afflicted nations like India and Thailand (Jackson et al. 2014; Viswanathan 2021; Li et al. 2024).

14.6.3.2 Smut

Particularly in China and other vulnerable areas, resistant cultivars improved by genomics-assisted breeding help decrease smut caused by *Sporisorium scitamineum*. According to recent studies, applying silicon (Si) can boost smut resistance by boosting pathogenesis-related enzymes, secondary metabolites, and defensive pathways, strengthening the plant's defenses against pathogen invasion (Singh et al. 2022; Song et al. 2022). The increased expression of two essential enzymes in the pathogen response, polyphenol oxidase and phenylalanine ammonia-lyase, is linked to this resistance. Deng et al. (2020) genomics study showed that these metabolic changes brought on by silicon are associated with a lower incidence of smut, providing a physiological and genetic foundation for combining Si control with breeding initiatives aimed at producing smut-resistant lines.

14.6.3.3 Mosaic Disease

Stunted growth and decreased sugar content result from mosaic disease, brought on by the sugarcane mosaic virus (SCMV). With the recent use of CRISPR/Cas9 for the targeted elimination of susceptibility genes, such as eIF4E, a gene implicated in viral transmission, genetic research has helped identify resistance loci (Tian et al. 2023). Together, this research demonstrates how genomics-assisted breeding techniques, such as QTL mapping, GWAS, and CRISPR technology revolutionize disease-resistance breeding in sugarcane. By improving the genetic knowledge required for the creation of resistance markers, these cutting-edge molecular approaches are hastening the introduction of robust cultivars against diseases that have a significant economic impact on the world's sugarcane sectors.

14.7 Stress Tolerance (Biotic and Abiotic)

Genomes-assisted breeding is essential to creating sugarcane cultivars resilient to biotic and abiotic stressors, which substantially influence sugarcane production and quality. This method increases the resilience of the crop by identifying and incorporating stress-tolerant genes into breeding programs using molecular tools.

14.7.1 Abiotic Stress Tolerance

Genes like DREB2A, AREB1, and NHX1 are essential for tolerance to abiotic stresses such as heat, salinity, drought, and osmotic pressures. Research has demonstrated that the transcription factors DREB2A and AREB1 are activated in response to water deficiency, improving sugarcane cells' ability to retain water and increasing drought tolerance (Kumari et al. 2022; Bhatt et al. 2023a, b). In a similar vein, NHX1 contributes to ion homeostasis, which confers resistance to salt stress. Extensive differential expression of these genes was found in hybrids such as Guitang08–1180 and ROC22, highlighting their significance in response to cold and drought stressors (Kumar et al. 2024; Viswanathan 2023).

14.7.2 Biotic Stress Tolerance

Biotic stress resistance aims to fend off threats from bacteria, fungus, and pests. Breeding efforts use genes like RPM1 and RPS2, which are linked to resistance against bacterial infections, to increase sugarcane's resilience to illness. A role in stress adaptation across biotic and abiotic difficulties is suggested by the gene Sspon.06G00100300, which has been linked to pathogen response and demonstrated notable upregulation under cold stress (Manimekalai et al. 2023). These genomics-assisted approaches incorporate cutting-edge molecular techniques like CRISPR for precise gene editing to create sugarcane cultivars that can flourish in challenging environmental circumstances while retaining high yield and quality (Song et al. 2021; Joshi et al. 2022). Building on these discoveries, researchers now use gene markers to target particular sugarcane stress-tolerance genes and expedite breeding procedures (Malviya et al. 2021).

14.7.3 Bioenergy Traits (Biomass and Fiber)

With an emphasis on increasing biomass yield and fiber content, which are the two crucial elements for biofuel production, genomics-assisted breeding has significantly impacted the development of bioenergy features in sugarcane. Although traditional breeding is difficult due to the genetic complexity of sugarcane, a polyploid crop, breakthroughs in genomics have made more focused methods possible. Genes associated with the cellulose and lignin biosynthesis pathways have been identified as key genes for improving bioenergy features. These genes directly affect biomass accumulation and cell wall structure, which is essential for bioethanol production. Genes such as CESA (cellulose synthase) and SUS (sucrose synthase) have been targeted to improve the energy yield per plant to increase biomass production and cell wall thickness to improve the energy yield per plant. Because CRISPR-based editing allows for precise changes in genes, like CAD (cinnamyl alcohol dehydrogenase), which controls lignin biosynthesis, it has successfully altered sugarcane biomass properties. Sugarcane is more suitable for the manufacture of bioethanol when the amount of lignin in its cell walls is reduced since this promotes enzymatic breakdown during the biofuel manufacturing process. According to Ahmed et al. (2024), sugarcane's biofuel efficiency can be significantly increased by modifying these genes using CRISPR without sacrificing growth (Ahmad and Ming 2024). Additionally, some researchers, such as De Souza et al. (2019), have demonstrated that lignocellulosic content may be optimized using gene knockdowns of particular cell wall biosynthesis genes, which maximizes biomass accumulation and digestibility—two important factors for bioenergy applications.

Another useful method for choosing high-biomass sugarcane varieties is genomic selection (GS). As demonstrated by studies employing extensive SNP arrays in commercial cultivars to predict bioenergy trait potential with considerable accuracy, breeders use GS to evaluate thousands of single nucleotide polymorphisms (SNPs) across the genome to predict traits like biomass and fiber content more accurately (Hayes et al. 2021). Furthermore, scientists can now map gene networks influencing biomass production under different environmental conditions thanks to developments in integrative genomics, which combines transcriptomics and proteomics. This helps to further guide selective breeding efforts (Zhang et al. 2022).

These techniques demonstrate how sugarcane's growth as a sustainable bioenergy crop has been expedited by genomics-assisted breeding. Researchers have developed a more effective method for breeding high-biomass, low-lignin sugarcane cultivars that may sustainably meet bioenergy demands by combining CRISPR editing with GS and functional genomics techniques.

14.8 Challenges and Limitations in Sugarcane Genomics

14.8.1 Polyploidy Complexity

Genomics-assisted breeding for sugarcane aims to maximize yield and structural composition while improving the crop's suitability as a bioenergy crop. Although sugarcane's polyploidy and high genomic complexity have presented considerable difficulties, recent developments in genetic tools and sequencing technology are assisting with techniques for improving traits. Researchers have focused on several genes and processes related to cell wall composition, lignin production, and carbohydrate partitioning to enhance fiber and biomass features. The alteration of lignin, which affects the effectiveness of biomass for biofuel, has been the subject of important genetic research. For example, studies on the gene for lignin synthesis, COMT (caffeic acid O-methyltransferase), revealed that targeted mutagenesis might lower lignin content in sugarcane while increasing saccharification efficiency, a desirable characteristic for biomass conversion. Kannan et al. (2018) showed notable lignin reductions using TALEN-mediated mutation approaches, which enhanced cell wall digestibility and increased biofuel yield. This demonstrates how sugarcane's biofuel potential can be improved through lignin-targeted changes that optimize its biochemical profile for simpler conversion (Kannan et al. 2018; Meena et al. 2020).

Increasing the amount of fiber by improving the genes controlling cell wall production has also been a focus. Vasantha et al.'s (2021) research emphasized the function of genes that regulate the buildup of cellulose in cell walls, such as SUS (sucrose synthase) and CesA (cellulose synthase). It has been shown that increasing the expression of these genes increases the amount of cellulose, which directly affects fiber strength and biomass yield. Additionally, efforts are made to improve the expression of PAL (phenylalanine ammonia-lyase), which involves the phenylpropanoid pathway, a key player in the formation of lignin and cell walls (Vasantha et al. 2021).

Optimizing biomass traits also heavily relies on epigenetic changes. A customized strategy to improve desired bioenergy features through genomic and epigenetic control is further made possible by research by De Souza et al. (2021) that shows DNA methylation patterns in polyploid plants, such as sugarcane, can impact the expression levels of important fiber-related genes. These gene-editing initiatives are now more accurate and effective thanks to developments in CRISPR/Cas9, which enable targeted modification of multiple gene copies in the polyploid genome of sugarcane.

In conclusion, targeted gene editing, metabolic pathway optimization, and utilizing polyploid complexity for advantageous results all contribute to the significant advancements in genomics-assisted breeding for bioenergy attributes in sugarcane. According to De Souza et al. (2019), these initiatives have opened doors to increasing sugarcane as a sustainable bioenergy source by using molecular advancements to increase biomass yield and quality.

14.8.2 Breeding Time Lag

The lengthy breeding cycle lag in sugarcane genomics is a significant obstacle to crop variety improvement at a rate that satisfies worldwide demand. Sugarcane breeding efforts are particularly lengthy because of the crop's distinct biological and genetic characteristics. Complex breeding requirements, such as several stages of selection and field testing to guarantee desired traits, like yield, disease resistance, and sugar content, are reliably expressed under various environmental conditions, and it can typically take 10–15 years to develop and release a new cultivar. Sugarcane's long maturity period and perennial nature slow down the trait assessment and selection processes, limiting genetic gain over time, in contrast to many crops where desirable traits may be evaluated relatively quickly (Meena et al. 2022).

The fact that sugarcane is polyploid—it can have anywhere from 100 to 130 chromosomes—makes things even more complicated since it creates significant genetic redundancy and necessitates complex selection procedures. Because of the intricate gene connections across several chromosomes, this polyploidy not only prolongs breeding times but also makes exact trait selection more difficult. The complexity of genomic selection efforts is increased for breeding programs by the requirement to precisely forecast the manifestation of selected traits in a polyploid setting (Trujillo-Montenegro et al. 2021; Aitken et al. 2020).

Technological techniques like genomic selection (GS) and marker-assisted selection (MAS) are being developed to overcome these obstacles. By removing candidates early in the breeding pipeline based on genetic data, GS, in particular, enables breeders to determine the breeding value of plants at an early stage, which could expedite the selection process and possibly shorten the breeding cycle. However, the high prices of genomic technologies present another obstacle for some projects, particularly those in resource-constrained countries, and their implementation necessitates significant computational resources and experience (Chen et al. 2023b; Meena et al. 2022). However, substantial funding and research are still needed to fully realize their potential in sugarcane genomics, continuous developments in bioinformatics and high-throughput sequencing hold promise for overcoming these obstacles and speeding up the breeding cycle.

14.8.3 High Costs and Technological Limitations

Expensive expenses and technological constraints severely hamper developing and implementing sophisticated breeding techniques in sugarcane genomics. The complexity of sugarcane's polyploid genome, which necessitates the use of complex and frequently expensive high-throughput sequencing and phenotyping technologies, is the primary cause of these financial and technological obstacles. Integrating contemporary genomics techniques, including transcriptomics, whole-genome

sequencing, and molecular marker technologies, necessitates extensive data analysis and storage needs, both of which need a lot of resources.

According to Luo et al. (2023), high-throughput phenotyping technologies require significant infrastructure investments, even though they are transformational. Although phenotyping instruments are crucial for evaluating yield-related features because they can record comprehensive information on plant growth and stress response, their high cost restricts their use to institutions with adequate funding. Additionally, the cost of genomics-assisted breeding programs is increased by the need for a trained workforce with knowledge of quantitative genetics and bioinformatics to execute genomic selection (GS) and other data-intensive breeding techniques (Luo et al. 2023).

Another aspect of the cost dilemma is the requirement for ongoing scientific advancements to stay competitive in creating durable, high-yield sugarcane cultivars. As technologies like synthetic biology and CRISPR develop, breeding programs have to make costly investments in modernizing lab equipment and procedures (Lu et al. 2024). In response, some researchers support international partnerships to pool resources and knowledge, allowing for greater access to cutting-edge breeding instruments and enhancing sugarcane breeding efficiency worldwide (Lu et al. 2024). These financial and technological obstacles must be overcome for sugarcane breeding to advance. Making genomics-assisted breeding more accessible and practical for a more extensive range of breeding projects would need efforts to democratize and streamline the use of genomic technologies, especially in developing nations where sugarcane is a vital crop.

14.8.4 Real-World Applications and Prospects

These functional genomics methods are revolutionizing sugarcane breeding because they enable direct modification of genes associated with resistance and production. They offer a potent supplement to conventional breeding by making it possible to identify and validate essential genes in economically significant features more effectively. Sugarcane breeding can become more accurate as these tools develop, especially when CRISPR and RNAi technologies are integrated. This will reduce the time and resources required to create improved varieties that sustainably support higher yields, withstand changing climates, and resist diseases. Several interrelated factors influence the plateau in sugarcane yield per hectare. Expanding cultivation onto marginal lands—areas less suited for agriculture—means that fields may require intensive resource inputs, such as irrigation and fertilizers, just to maintain yields. These regions often have less favorable soil conditions, limiting crop productivity.

In addition, mechanical harvesting, although labor-saving and efficient, can impact soil structure and quality over time. Frequent machine traffic on the fields can lead to soil compaction, reducing water infiltration and root growth, ultimately

diminishing soil health and crop yields. This compaction also worsens with repeated cultivation cycles, especially in regions with minimal soil management practices.

Long-term soil degradation from repeated sugarcane planting is another critical factor. Sugarcane is a nutrient-demanding crop, and continuous cultivation without adequate soil replenishment can lead to nutrient depletion and a decrease in organic matter. Over time, this decline in soil fertility can create conditions where yields plateau unless further intensification of inputs occurs—though this is often economically and environmentally unsustainable. In sum, while mechanization and expansion into marginal lands may have initially supported the growth of the sugarcane industry, they likely contributed to the productivity plateau due to the increased need for costly inputs and the long-term degradation of soil health.

14.9 Future Prospective

One of the most promising techniques in sustainable sugarcane breeding is genomic selection (GS). GS can enhance the accuracy of predicting beneficial traits at the seedling stage, which shortens the breeding cycle and enables the early selection of superior clones. This rapid cycle selection can increase genetic gains for yield, disease resistance, and resilience to environmental stresses, which reduces dependency on fertilizers and pesticides. For example, a study by Yadav et al. (2020) demonstrated that GS methods could enhance genetic gains up to two-fold compared to traditional breeding, effectively supporting sustainable production through higher resource efficiency (Voss-Fels et al. 2021). Incorporating wild relatives, such as *Saccharum spontaneum*, enhances tolerance to drought and nutrient-poor soils, crucial traits under climate stress. Genomic technologies enable breeders to incorporate diverse alleles from wild relatives more effectively, creating varieties that require less water and are more nutrient-efficient. This approach helps reduce the need for synthetic fertilizers, minimizing environmental impact (Deomano et al. 2020; Yadav and Singh 2021).

Disease resistance is another focus of genomic research in sugarcane, given the crop's susceptibility to fungal infections that traditionally necessitate chemical interventions. GS allows breeders to select clones with inherent resistance to diseases like rust and smut early in the breeding process. These disease-resistant varieties reduce the need for fungicides, directly contributing to lower chemical inputs and promoting environmental health. Researchers have applied genomic selection for brown and orange rust resistance, achieving high prediction accuracies and promising for wider application in commercial breeding programs (Islam et al. 2021). Potential Sugarcane's role as a bioenergy crop has spurred research into improving its biomass yield and sugar content through genomics. Developing high-biomass, low-input varieties can significantly enhance bioethanol yields without increasing the carbon footprint, supporting cleaner energy production. Genomic studies have identified key quantitative trait loci (QTL) that impact both cane yield and sucrose content, allowing breeders to produce varieties optimized for biofuel,

which contributes to reducing fossil fuel reliance and carbon emissions (Cursi et al. 2021).

With climate change affecting agricultural productivity, sugarcane breeding focuses on traits like drought tolerance, deeper root systems, and heat resistance. Molecular breeding tools, such as genome-wide association studies (GWAS) and SNP (single nucleotide polymorphism) markers, help identify genetic factors associated with these traits. Such climate-resilient varieties can maintain productivity under extreme weather conditions, reducing the likelihood of crop failure and the need for emergency inputs. In sum, integrating genomic and molecular breeding techniques into sugarcane breeding programs can revolutionize sustainability in sugarcane production. Through increased genetic gain, reduced chemical reliance, and enhanced climate adaptability, genomics and molecular breeding provide tools to develop sugarcane varieties that are both high yielding and aligned with sustainable agricultural practices.

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Chapter 15
Genomics-Assisted Breeding: A Revolutionary Approach for Sugarcane Development

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Abstract Sugarcane (*Saccharum* spp.) is an economically important crop for the food and biofuel industries. However, its production faces significant challenges due to the changing climate and the growing global population. Traditional breeding approaches for sugarcane varietal development typically require 12–14 years. Additionally, precise phenotyping in sugarcane is challenging due to its susceptibility to lodging and suckering, which are influenced by environmental factors and management practices. To overcome these challenges, modern biotechnological approaches are crucial for enhancing sugarcane productivity. Recent advancements in sequencing technologies provide new opportunities to reveal genetic variation in sugarcane and enhance conventional breeding through genomics-assisted breeding

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(GAB). Integrating conventional breeding with marker-assisted selection and genomic selection (GS) using high-density SNP markers can accelerate the breeding process, resulting in improved genetic gains. GAB is becoming increasingly effective due to advancements in large-scale mapping of quantitative trait loci, gene cloning, elite allele mining, and GS. As more comprehensive genomic datasets emerge, GAB is expected to accelerate crop improvement efforts significantly. This chapter explores the latest advancements in GAB in sugarcane and explored future directions for this promising approach.

Keywords Sugarcane · Genomic assisted breeding · Genomic selection · Sequencing · SNPs

15.1 Introduction

Sugarcane (*Saccharum* spp.) is a globally significant crop, contributing approximately 80% of the world's sugar production and a substantial share of bioethanol used as a renewable energy source (Verma et al. 2024a, b). In 2021, global sugarcane production exceeded 1.96 billion tons, with 27 million hectares of land dedicated to cultivation across farms and estates ranging from a single hectare to thousands. The top producers were Brazil, with 752 million tons (36% of which was used for sugar and the rest for ethanol), followed by India at 405 million tons, Thailand at 131 million tons, and China at 110 million tons. Together, these four countries accounted for over 70% of the global sugarcane output (https://www.cirad.fr/en/content/download/9499/file/Sugarcane%20roadmap%20summary.pdf). With a rising global population and increasing demand for sugar and biofuels, it is essential to further improve sugar recovery and cane yields by 2050 without expanding the production area (Jaiswal et al. 2023; Chen et al. 2024a, b). Additionally, sugarcane faces numerous biotic and abiotic stresses that threaten productivity. Major biotic threats include diseases, such as sugarcane mosaic virus, brown rust (*Puccinia melanocephala*), and leaf scald (*Xanthomonas albilineans*), all of which can cause substantial yield losses (Verma et al. 2020a, b, 2021a, 2024c, d; Mehdi et al. 2024). For instance, the sugarcane yellow leaf virus (ScYLV) adversely affects yield and juice quality. Abiotic stresses, including drought, extreme temperatures, and poor soil fertility, further exacerbate growth challenges, affecting both yield quantity and quality and leading to economic losses for farmers and the industry (Verma et al. 2019a, b; Krishna et al. 2022; Jagadesh et al. 2024; Mehdi et al. 2024).

Advances in sugarcane breeding have significantly improved agronomic traits and crop yields. However, the increasing global demand for sugar and biofuels and climate change underscore the need for enhanced productivity in both cane and

sugar yields (Song et al. 2019; Malviya et al. 2020; Guo et al. 2022; Verma et al. 2022a, b). Achieving these genetic gains through conventional breeding methods is challenging because many agronomic traits are complex and polygenic, with each gene contributing a small effect (Singh et al. 2021; Wu et al. 2022). The highly heterozygous and autopolyploid sugarcane genome complicates the creation of a detailed genetic map. Additionally, the complexity of the sugarcane genome, coupled with its autopolyploid nature, makes it challenging to improve adaptability and yield (Singh et al. 2021a, b; Song et al. 2022; Joshi et al. 2023). This is due to multiple sets of homologous chromosomes, which complicates genetic analysis and the selection of beneficial traits. Despite these challenges, key agronomic traits for enhanced sugarcane production and resistance to diseases and pests have been identified using quantitative trait loci (QTL) mapping, genome-wide association studies (GWAS), and transcriptome analyses (Fickett et al. 2019; Li et al. 2023a, b; Lu et al. 2023). The reference genome of the cultivar "R570" has provided a valuable foundation for understanding its genetic architecture and identifying QTL linked to important agronomic traits (Malviya et al. 2021; Healey et al. 2024). While improvements are more straightforward for traits controlled by one or two genes, polygenic traits governed by multiple QTLs, each with small and variable effects, are more challenging to enhance through traditional breeding. Genetic drift often complicates these efforts, disrupting the assembly of desirable gene combinations (Singh et al. 2019; Chen et al. 2020; Meena et al. 2022; Afghan et al. 2024). Recent advancements in genomic selection (GS) have improved breeding efficiency by enabling researchers to select and accumulate desirable alleles linked to superior traits. GS leverages genomic estimated breeding values, significantly boosting selection accuracy and genetic gains in sugarcane breeding (Singh et al. 2020; Guo et al. 2021; Liang et al. 2024). Additionally, next-generation sequencing and genome-editing technologies are opening new pathways to optimize the sugarcane genome for favorable traits like an erect canopy, ideal leaf angle, prolonged greening, high biomass, deep root systems, and non-flowering characteristics (Anas et al. 2021a, b; Song et al. 2021; Malviya et al. 2024). Using these tools, researchers have explored valuable yield traits such as single cane weight, tillering capacity, millable cane numbers, and sugar quality attributes like sucrose and sugar yield (Verma et al. 2020c, d; Meena et al. 2022; Bhatt et al. 2024; Li et al. 2024a, b).

This chapter explores recent advancements in genomic approaches utilized in sugarcane breeding to address the challenges of improving complex traits. It covers modern techniques, such as GWAS, GS, and marker-assisted selection (MAS), which have revolutionized breeding by enabling more precise and efficient selection of desirable traits. Additionally, the chapter delves into high-throughput sequencing, transcriptomics, and bioinformatics tools that aid in understanding the genetic architecture of sugarcane (Verma et al. 2024a, e). Together, these approaches offer valuable insights and practical strategies for accelerating the development of high-yielding, resilient sugarcane varieties.

15.2 Genetic Resources and Its Utilization in Sugarcane Breeding

Genetic resources play a crucial role in the breeding and improvement of sugarcane, particularly in the face of various biotic and abiotic stresses that challenge its cultivation. The genus *Saccharum*, which includes several species such as *Saccharum officinarum*, *S. spontaneum*, and *S. robustum*, provides a rich pool of genetic diversity that can be harnessed for breeding programs. This diversity is essential for developing resilient varieties to diseases, pests, and environmental stresses like drought and waterlogging (Verma et al. 2020e, f, 2022c, d). The systematic collection and characterization of sugarcane germplasm, particularly in gene banks like the one in Kannur, Kerala, India, have resulted in a vast repository of accessions that breeders can utilize to enhance traits. Additionally, the United States Department of Agriculture (USDA), Agricultural Research Service (ARS) in Miami, maintains one of the largest global sugarcane germplasm collections, known as the World Collection of Sugarcane and Related Grasses—different genomic and genetic resources represented in Fig. 15.1 for sugarcane improvement (Afghan et al. 2024).

Using these genetic resources in sugarcane breeding has led to significant advancements in crop improvement. Breeders have successfully employed interspecific and intergeneric hybridization techniques to combine desirable traits from wild relatives with cultivated varieties (Santchurn et al. 2019; Sharma et al. 2021). For instance, *S. spontaneum* has been instrumental in imparting resistance to red rot and other diseases, while *Erianthus* species have contributed to traits like drought tolerance and biomass yield. The ongoing efforts to screen and evaluate these genetic resources ensure that breeders can continuously identify and incorporate new sources of resistance and adaptability into their breeding programs (Huang et al. 2022; Xu et al. 2022; Deng et al. 2023; Tian et al. 2023). This strategic utilization enhances the resilience of sugarcane varieties and supports sustainable agricultural practices by improving productivity and reducing the reliance on chemical inputs (Verma et al. 2021b, c; Huang et al. 2024). Numerous mapping populations have been developed using available resources to identify key genomic regions and produce improved cultivars. For instance, in QTL mapping studies, 285 F_1 progeny were generated from a cross between *Yuetang 93-159*, a moderately resistant variety, and *ROC22*, a highly susceptible variety, through artificial infections with a mixed inoculum of sugarcane mosaic virus (SCMV) and sorghum mosaic virus (SrMV). The analysis identified 29 resistant, 55 highly resistant, 70 moderately resistant, 62 susceptible, and 40 highly susceptible progenies (Lu et al. 2023). Additionally, phenotypic data from these 285 F1 progenies showed significant differences in tillering rate for the *YT93-159* × *ROC22* cross (Wang et al. 2023). Another study assessed an F_1 segregating population from a cross between two hybrid clones, *CP95-1039* and *CP88-1762*, for orange rust resistance (Yang et al. 2018). Collectively, these studies provide a robust foundation for genetic improvement in sugarcane (Sharma et al. 2022; Chen et al. 2023a, b).

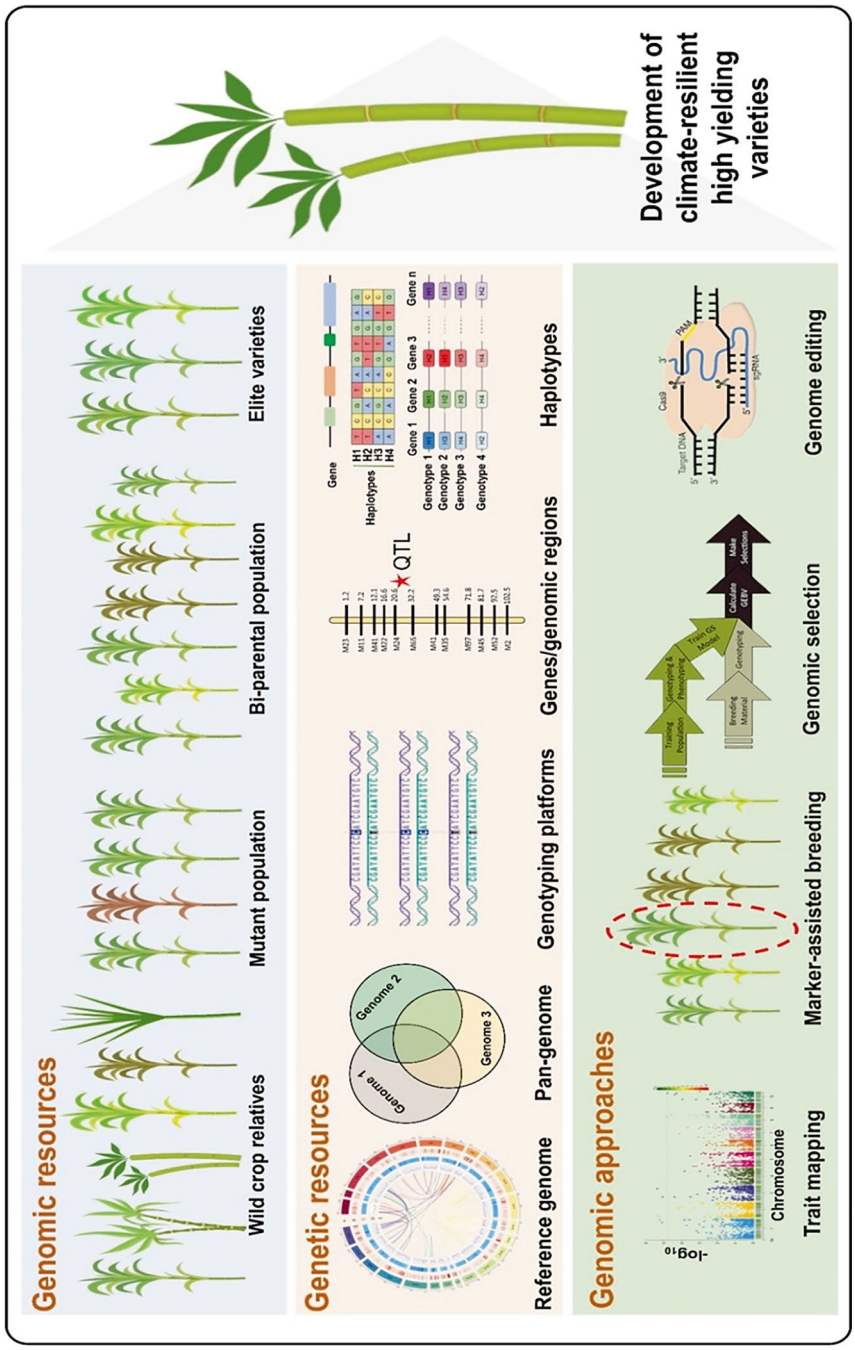

Fig. 15.1 Utilizing genomic and genetic resources for sugarcane improvement: An overview of diverse genetic approaches for enhancing yield, disease resistance, and stress tolerance

15.3 Complexity of the Sugarcane Genome

The complexity of the sugarcane genome, characterized by high ploidy levels and considerable hybridization, has posed substantial challenges to genetic research and breeding programs. The complexity of the sugarcane genome is fundamentally linked to its polyploid nature, with modern cultivars generally exhibiting 10–12 homologous chromosome copies, resulting in a total chromosome count ranging from 100 to 130 (Healey et al. 2024; Thirugnanasambandam et al. 2018). The *Saccharum* genus comprises six primary species: two wild species, *S. spontaneum* (2n = 40–128, x = 8) and *S. robustum* (2n = 60–80), and four cultivated species, *S. officinarum* (2n = 80, x = 10), *S. barberi* (2n = 81–124), *S. sinense* (2n = 111–120), and *S. edule* (2n = 60, 70, 80). The sugarcane genome is significantly large, comprising approximately 8.7 billion base pairs (R570 cultivar), making it about three times larger than the size of the human genome and 20 times larger than that of rice (Healey et al. 2024). This large genome size is further complicated by multiple homologous chromosomes with multiple alleles, making the assembly and analysis challenging. This large and complex genome demands extensive molecular markers to ensure comprehensive genomic coverage in sugarcane (Gouy et al. 2013).

As a result, identifying markers associated with desirable traits becomes difficult in sugarcane breeding programs. Moreover, the process of selecting superior F_1 hybrids with desirable alleles is hindered by the significant random segregation of homologous and homologous chromosomes, which often leads to the generation of recombinant genotypes (Grivet and Arruda 2002). Thus, recent genomic initiatives have aimed to construct a comprehensive reference genome for sugarcane by sequencing diverse cultivars and identifying protein-coding genes involved in sucrose and starch metabolism. (De Setta et al. 2014). The complex sugarcane genome, rich in transposable elements and structural variations, hinders traditional breeding. However, it enables advanced GS techniques that leverage genome-wide markers to accelerate breeding and improve genetic gain by capitalizing on the full allelic diversity of sugarcane germplasm (Mahadevaiah et al. 2021; Kandel et al. 2018). As a result, genomic resources are increasingly essential for improving sugarcane traits such as disease resistance, biomass yield, and sugar content, thereby supporting the crop's role in sustainable agriculture and bioenergy production (Verma et al. 2021d, e, 2023a, b).

15.4 Genomes and Pan-Genome for Sugarcane Breeding

Genomic resources have expanded tools for plant breeding and crop improvement. Early plant genome assemblies in the 2000s transformed our understanding of crop biology, particularly revealing diversity at the SNP level. The sugarcane genome is highly complex due to its polyploid nature, resulting from the hybridization of *Saccharum officinarum* and *Saccharum spontaneum*. This complexity, with modern

cultivars containing between 100 and 130 chromosomes from both species, poses challenges for genome assembly and annotation. Sequencing the haploid *S. spontaneum* (AP85–441) enabled the assembly of 32 pseudo-chromosomes, organized into 8 sets, with 35,525 genes and their alleles identified (Zhang et al. 2018; Verma et al. 2022e, f). The randomized distribution of *S. spontaneum* chromosomes in modern cultivars suggests diverse recombination patterns across accessions. Advances in sequencing, especially long-read sequencing, have recently enabled high-quality assemblies, such as the chromosomal-scale assembly of hybrid cultivar ZZ1, which spans 10.4 Gb with 68,509 annotated genes (Bao et al. 2024). These assemblies are invaluable for gene identification, particularly those linked to key agronomic traits.

Single-reference genomes capture only a limited portion of a species' full genetic diversity, prompting the development of pan-genomes to better represent this variability. Structural variants (SVs), including presence–absence variations (PAVs) and copy number variations (CNVs), play a significant role in differences within genome content. The "core" genome encompasses genetic material shared by all individuals, while variable sections comprise the "dispensable" genome (Jha et al. 2022). To date, a pan-genome has not yet been developed for sugarcane. Constructing a pan-genome for this crop would be highly valuable, as it would capture the full spectrum of genetic diversity across different sugarcane varieties. This comprehensive resource would support the identification of unique structural variations. A sugarcane pan-genome would ultimately enhance breeding efforts by providing breeders with more precise genetic information to select and improve desirable traits.

15.5 Haplotype and *k*-mer-Based Breeding

Haplotype and *k*-mer-based breeding represent advanced genomic techniques offering significant advantages in crop improvement by enhancing the efficiency and accuracy of GS. A haplotype refers to a specific combination of alleles corresponding to different polymorphisms (i.e., single nucleotide polymorphisms, insertions/deletions, and other genetic markers) located on a single chromosome, which are transmitted together with a minimum probability of contemporary recombination (Stram 2017; Garg 2021). Haplotype-based breeding facilitates the identification of superior alleles linked to desirable traits, simplifying the selection process by reducing the complexity of genetic data by aggregating SNPs into haplotype blocks. This approach improves predictive capabilities in breeding programs and reduces the likelihood of false associations, thus accelerating the development of resilient crop varieties under fluctuating environmental conditions (Verma et al. 2022g; Singh et al. 2024; Sivabharathi et al. 2024).

This strategy has demonstrated an enhancement in the precision of genomic predictions and the utilization of hybrid vigor in crops such as tea and potatoes, where heterozygosity is pivotal in the manifestation of phenotypic traits (Zhang et al. 2021; Sun et al. 2022; Solanki et al. 2023; Kumari et al. 2023). Liu et al. (2023)

discovered 39 SPSB (sucrose phosphate synthase B) haplotypes from 18 sugarcane varieties linked with higher sucrose accumulation, each spanning 2824 base pairs. These haplotypes were primarily located on chromosomes Chr3B, Chr3C, and Chr3D, according to the AP85–441 reference genome. The study revealed numerous genetic variations, including SNPs and insertion/deletion (InDel) changes, with an average of 42 base pairs per variation. Six haplotypes (HAP4, HAP6, HAP7, HAP9, HAP17, and HAP20) were identified as positively selected, suggesting a link to elevated sucrose synthesis efficiency. The findings indicate that the mutations in SPSB haplotypes in high-sucrose clones are favorable and may be selected and fixed during breeding processes. Furthermore, Zhang et al. (2013) conducted haplotype analysis of the *SuSy* (sucrose synthase) gene family in three *Saccharum* species, elucidating genetic diversity and functional significance in sugarcane. Five SuSy genes were recognized *in S. officinarum, S. robustum, and S. spontaneum,* yielding 27, 35, and 32 haplotypes, respectively. This research offers critical insights for formulating sequencing strategies for sugarcane's complex autopolyploid genomes, which are vital for breeding efforts to enhance sugar and biomass production (Singh et al. 2023; Guo et al. 2023).

However, *k*-mer-based methods analyze genetic variation by utilizing short DNA sequences (*k*-mer) without relying on a reference genome, streamlining the identification of beneficial diversity across populations (Karikari et al. 2023). A k-mer is a sequence of *k*nucleotides (bases) of a fixed length extracted from a more extensive DNA sequence and widely used to analyze genetic sequences, especially for genome assembly, sequence alignment and detection of variation (Sievers et al. 2017; Oliveira et al. 2022; Aylward et al. 2023). This method is invaluable for assessing population diversity and facilitating GWAS by detecting single nucleotide polymorphisms (SNPs) and structural variations (Audano et al. 2018; Singh et al. 2022; Aylward et al. 2023).

In a study conducted by Grativol et al. (2014), methylation filtration (MF) was used to improve sugarcane genomic assembly by filtering out repetitive sequences, leading to a 134-fold increase in genic coverage. This study optimized the k-mer size of 49, which consequently discovered 291 SNPs linked to the sucrose/starch pathway, offering significant insights for breeding programs focused on enhancing sugar content and biofuel potential in sugarcane. Additionally, Pompidor et al. (2021) conducted a haplotype analysis in sugarcane by analyzing SNPs in specific genomic regions (*Adh1* and *Rpa1*). The study identified three distinct haplotypes, that is, A and B, mainly derived from the domesticated *S. officinarum* and its wild ancestor *S. robustum,* while haplotype C originated from *S. spontaneum.* This differentiation was substantiated by unique SNPs for each haplotype group, with 97, 65, and 86 SNPs identified for haplotypes A, B, and C, respectively. In both studies, k-mer analysis played a crucial role in identifying orthologous regions through filtration of sequence data based on common k-mers, which facilitated the mapping of reads to the reference genome and the subsequent identification of SNPs. The integration of these two approaches allowed for a deeper understanding of modern sugarcane cultivars' genetic diversity and evolutionary history, ultimately leading to

improved sugarcane cultivars with enhanced yield and resilience (Malviya et al. 2022; Zheng et al. 2023).

15.6 Genomic Selection (GS) to Accelerate Genetic Gain

Conventional phenotypic selection relies on easily observable traits, which are highly inefficient as the expression of agronomic traits is complex and is affected by environmental conditions owing to a high G × E interaction magnitude. Also, developing promising varieties takes a very long time, leading to lower genetic gain (Li et al. 2023a, b; Anand et al. 2023). Incorporation of biochemical, cytological and molecular markers can enhance genetic gain through factors such as high heritability and less time demand (Siddique et al. 2024). Identification of genomic regions linked to the quantitative traits through the QTL mapping followed by the introgression of loci to develop genotypes with desirable traits with the help of markers tightly linked to that loci known as marker-assisted selection (MAS) is a reliable and less time-consuming method as compared to conventional phenotypic selection (Collard and Mackill 2007).

In 1986, Beckmann and Soller first used the term "marker-assisted selection (MAS)" (Beckmann and Soller 1986), which demands the prerequisite of genetic markers tightly linked to the validated QTLs controlling that trait, which is rare in practical scenarios. In addition, MAS has some severe drawbacks, such as linkage drag, the genetic background of the recipient genotype, and, most importantly, it cannot operate with highly complex quantitative traits such as abiotic stress tolerance and yield (Sharma et al. 2024). GS is a specialized type of MAS, and the significant differences are presented in Table 15.1. GS, however, gives account to the minor QTLs contributing to the traits, hence facilitating the improvement of highly complex traits and saving the cost of phenotyping for the breeding population, especially for traits such as abiotic stress tolerance and yield, and for traits for which phenotyping is very expensive and time-consuming, such as quality traits and biochemical traits (Bhatt et al. 2023a, b; Xu et al. 2024; Kumari et al. 2024).

GS is one of the most recent methodologies based on high-throughput data that significantly accelerates genetic gain (Xu et al. 2020) by accounting for individual marker effects densely distributed throughout the genome of species proposed by Meuwissen along with coworkers in 2001 (Meuwissen et al. 2001). Under this scheme, the sole effect of each marker on phenotype is estimated. Using this, an additive sum of all the markers is used for the genomic prediction of superior genotypes (Budhlakoti et al. 2022). The generalized scheme for the GS approach starts with constructing a training population with both phenotyping and genotyping data; in contrast, a breeding population for which only genotypic data is generated. The data from the training and breeding population is used to build a GS model that utilizes phenotypic data as the effect and genotypic data as the cause, and by correlating this, it yields genomic estimated breeding values (GEBV) of individual lines (Tade and Melesse 2024). By following this methodology, GS offers a drastic

Table 15.1 Key Differences between marker-assisted selection (MAS) and genomic selection (GS)

Feature	MAS	GS
Selection scheme	Indirectly through the genetic markers tightly linked to the validated QTL.	Based on GEBV values calculated through a statistical model built over data provided by training and breeding populations.
Trait improved	Only oligogenic or traits governed by major QTL with a significant effect on trait expression can be improved.	It accounts for all major and minor QTLs; complex quantitative traits can also be improved.
Prerequisite	Validation of QTL related to trait and screening of markers for linkage with QTL.	Building GS model based on data provided by training and breeding set.
Principle	It is highly specific toward the selection of introgressed QTL through tightly linked markers.	The comprehensive approach that addresses all the markers used during the building of the GS model.
The magnitude of genetic gain per unit of time	Less, due to high time consumption during QTL validation and marker screening.	High, as it does not require QTL validation and marker screening also saves the time needed for the phenotyping of the breeding population.
Genome coverage	It can be specifically utilized either for the selection of desired introgression or elimination of undesirable genomic regions.	It can cover the whole genome through the markers distributed throughout the genome.
Limitations	Linkage drag, effect of genetic background and cannot be used for minor QTLs.	Factors affecting the prediction ability of GS.

decline in the breeding cycle by eliminating cost, labor, and time associated with extensive phenotyping (especially in the case of quality traits and phenotyping for stress tolerance); in contrast to MAS, it gives an account for markers distributed across the genome. Due to these advantages in the current era of climate change, GS is gaining popularity as a promising tool among breeders to develop abiotic stress-tolerant genotypes.

Incorporation of multi-environment (location/year combinations) while genomic prediction for trait performance in non-phenotyped individuals (breeding set) increases the complexity of GS models and causes a decline in the prediction ability (PA) of the model. Statistical models can operate for multiple traits and environments and give an appropriate account for the complexity of genetic control of traits and the interaction between the genetic loci controlling that trait and genetic background (Kumari et al. 2022; Chiaravallotti et al. 2024). Intensive research is directed toward developing more accurate parametric and semiparametric models for genomic prediction. Different practices have been developed for GS to forecast GEBVs precisely in several crops. These comprise nonlinear semi-parametric techniques such as reproducing kernel Hilbert space (RKHS), linear parametric methods such as ridge-regression best linear unbiased prediction (rrBLUP), nonlinear

non-parametric techniques (RF), Bayesian techniques (BL), and machine learning techniques. All of these techniques have proven effective in precisely estimating GEBVs. These days, plant-breeding programs frequently use the genomic best linear unbiased prediction (GBLUP) model. By mimicking actual forecasting situations, genomic prediction models address a variety of prediction complications. Many random cross-validation techniques have been developed to meet the prediction issues associated with GS.

Particularly in sugarcane, GS for smut resistance (*Sporisoriums citamineum*) showed that marker selection with attention network analysis yielded higher PA also reduced the computation time, as well as the inclusion of BayesR and GWAS-derived markers, which gave better PA (Chen et al. 2024a, b). Though it varies from model to model, generally, training population size and marker density have a direct relationship with PA up to a specific limit, after which an increase in population size, as well as marker density, does improve PA by a negligible margin (Islam et al. 2021). Genomic prediction (GP) for ratooning ability resulted in a higher PA for economic profitability than any other trait in the additive dominance epistasis (ADE) model as compared to other models. Also, the fluctuations in PA among different models were found to be comparative (Islam et al. 2023). GS exhibits superior performance over MAS as the number of simulated quantitative trait nucleotides (QTNs) and complexity of genetic architecture increases thus, GS is favored for its broader applicability and higher accuracy in complex genetic environments, highlighting its superiority over MAS for predicting trait values in varying genetic architectures (Olatoye et al. 2019).

Utilization of trait-specific markers had superior results for PA for sucrose and fiber content traits, which suggests that the GS approach could perform a strategic role in the improvement of these traits (Verma et al. 2023c, d; Xiong et al. 2023). Better PA was achieved through an extended GBLUP model as compared to the additive GBLUP for tons of cane per hectare (TCH), and optimization of the model by incorporating the larger size of training population size resulted in improvised PA for CCS, TCH, and fiber (Chen et al. 2023a, b). For traits such as TCH and CCS, RKHS and AK4 models outperform owing to the non-additive action, whereas for fiber, the GBLUP model uniformly performed well due to high heritability. A smaller sample size combined with genetic heterogeneity can lower the PA, and the choice for SNP parametrization can have negligible influence on PA through the genetic relationship assessment (Yadav et al. 2024). Improvement in the PA with elevation in marker density was marginal up to a plateau, whereas PA increased gradually with higher training set (TS) size, so it can be inferred that TS size has a profound effect over marker density, irrespective of the TS size. Bayes A, Bayes B, and RKHS models yielded higher PA for the majority of the yield and quality-determining primary and secondary traits of sugarcane (Joshi et al. 2022; Islam et al. 2022)**.** Addressing environmental conditions while building a model can enhance the prediction accuracy, inferred by an increase in PA by incorporating soil and plant type as fixed effects caused by an increment in the PA (Satpathy et al. 2022).

15.7 Trait Mapping to Uncover Genomic Regions

Conventional breeding methods have led to the generation of numerous sugarcane varieties. However, these approaches lag behind the demand for increased yield performance and improved biotic and abiotic stress tolerance. As we can see, there is a tremendous shift toward an effective understanding of the intricate relationships between various important traits and genotypes by exploring diverse methodologies. Among these methods, QTL mining techniques, such as QTL mapping and association mapping, have gained importance (Gangurde et al. 2022; Xu et al. 2017). The difference between QTL mapping and association mapping (Table 15.2). Even if QTL mapping is fundamental and the most widely used approach for marker identification, it has many setbacks, viz., the development of mapping populations is tedious, the generation of a single population segregating for multiple traits is complex, and there is a lack of high-density mapping (Varshney et al. 2005).

In the last three decades, numerous examples of linkage analysis have been reported in sugarcane. GWAS is a more robust and promising approach, having higher resolution, whole genome coverage, higher allelic frequencies, and the ability to detect historical recombination events. With the advancement in high-throughput genotyping and sequencing technologies, the GWAS tool is turning out to be very promising for detecting genes having quantitative trait variation (Naik et al. 2024). GWAS helps identify links between genetic variants and specific traits, offering higher precision than traditional QTL mapping. GWAS works by analyzing genotypes and phenotypes to find associations. Although standard mixed linear

Table 15.2 Comparison of QTL mapping and genome-wide association studies (GWAS)

Parameters	QTL mapping	GWAS
Study population	Structured populations such as F_2, backcross (BC), recombinant inbred lines (RIL), or double haploids (DH).	Natural or diverse populations, such as groups of unrelated individuals or populations with varying levels of genetic relatedness.
Markers	Fewer markers (typically hundreds of thousands).	Millions of markers (usually SNPs).
Resolution	Low: Identifies broad regions of the genome.	High: Pinpoints specific loci or even single genetic variants.
Population type	Controlled, related populations (e.g., offspring of a cross).	Natural, unrelated populations.
Required sample size	Smaller sample size.	Larger sample size.
Mapping approach	Relies on genetic recombination during meiosis	Relies on linkage disequilibrium in natural populations
Time and cost	Time-consuming due to the need for controlled crosses.	It is less time-consuming but requires large datasets and computing power.
Typical outcomes	Identifies broad QTL regions potentially containing multiple genes.	Identifies specific SNPs or loci associated with traits.

models (MLM) are typical for GWAS, they can be slow when handling large datasets. Multi-locus models have been developed to address this. While SNP-based GWAS is widely used, it may miss weak signals or interactions between related SNPs (Fang and Luo 2019).

To overcome this, haplotype- and gene-based GWAS methods have been introduced, providing higher statistical power, especially for rare genetic variants (Alseekh et al. 2021). The amount of linkage disequilibrium (LD), which plays a significant role in the GWAS studies, is high in sugarcane, and this can be advantageous as compared to the traditional bi-parental mapping for the identification of the molecular markers of interest (Fickett 2018). This high LD results from the relatively limited number of generations between modern cultivars and the fewer clones used in the original hybridization. Aside from transgressive segregants, biparental populations experience a restricted number of recombination events, which can lead to larger linkage blocks (Gouy et al. 2015). Detecting a QTL within a large linkage block would be challenging, if not impossible, unless there is a significant trait effect (Zhu et al. 2008). In contrast, an association panel (a diversity panel) offers more significant genetic variation and smaller linkage blocks.

More advanced statistical techniques, such as the Anderson–Darling test, help identify genetic regions with modest effects, striking a balance between statistical power and false positives (Yang et al. 2014). While early GWAS studies raised concerns about missing heritability, larger datasets and more SNPs have increased the discovery rate for complex traits (Manolio et al. 2009). With the advancements in sequencing technologies in the last few decades, GWAS has been continuously expanded. GWAS has allowed us to study many plant traits simultaneously. There are various methods and tools used for analysis in this technique (Susmitha et al. 2023).

MLM frameworks in GWAS significantly reduce false-positive rates by accounting for population structure and kinship (Pritchard et al. 2000). Tools such as FaST-LMM (factored spectrally transformed linear mixed models) and EMMA (efficient mixed-model association) optimize MLM computationally, while SUPER (settlement of MLM under progressively exclusive relationship), CMLM (compress MLM), and MLMM (multiple-locus linear mixed model) improve statistical power. FarmCPU (fixed and random model circulating probability unification) further enhances performance by separating fixed and random effects, and BLINK (Bayesian information and linkage disequilibrium iteratively nested keyway) replaces the random-effect model with a fixed-effect model, improving efficiency for large datasets (Susmitha et al. 2023; Kumar et al. 2024).

In recent years, there has been tremendous growth in machine learning methods in GWAS. The approaches used in this method are regression, classification, ensemble-based learning, and neural networks. The logistic regression and the least absolute shrinkage and selection operator (LASSO) regularization approach are useful for GWAS. This approach overcomes many limitations of the MLM method (Susmitha et al. 2023). Classification methods based on support vector machines (SVM), such as COMBI, have been created to predict unknown phenotypes from

unseen genotypes (Mieth et al. 2016). A convoluted neural network (CNN) model was developed by Liu et al. (2019) to predict phenotype using the SNP dataset.

The genome-wide association studies (GWAS) in sugarcane crop has become popular in the last decade (Table 15.3). The GWAS has been used to identify the alleles from the germplasms which were related to resistance to numerous diseases like red rot (Singh et al. 2016; O'Connell et al. 2022), smut and leaf scald (Wei et al. 2006), sugarcane yellow leaf virus (SCYLV) (Pimenta et al. 2021), and also for other traits such as agro-morphology, sugar yield, bagasse content, polarization (Pol), and sugar yield (Ukoskit et al. 2019); sucrose traits viz., brix, total recoverable sugar (TRS), and percent sucrose (Fickett et al. 2019); fiber content (Xiong et al. 2023); drought tolerance (Wirojsirasaket al. 2023); yield-related traits (Zhang et al. 2023, Barreto et al. 2019). Furthermore, Dijoux et al. (2024) studied Sugarcane Orange Rust (SOR) resistance in 568 sugarcane hybrids using 183,842 SNPs through SL-GWAS, identifying five QTLs (Oru1–Oru5) from *S. spontaneum.* An additional, less significant QTL (*Oru6*) from *S. officinarum* was detected via ML-GWAS. Li et al. (2024a, b) conducted a GWAS on leaf scald disease in sugarcane using 170 genotypes and a 100 K SNP array. The study identified eight SNPs in multi-environment and five SNPs in single-environment analyses, utilizing GLM, MLM, MLMM, SUPER and BLINK models. These findings highlight genetic markers linked to leaf scald resistance.

Zhang et al. (2023) evaluated seven yield-related traits in 159 genotypes from various sugarcane species. They identified 47 SNPs linked to Brix across 36 QTLs and 138 SNPs for other traits across 104 QTLs. Additionally, they discovered 12 stable QTLs for yield traits comprising 35 candidate genes. Wirojsirasak et al. (2023) conducted a large-scale candidate gene association study on agronomic traits and drought tolerance in sugarcane, using 649 candidate genes. They identified 197 significant MTAs across 18 evaluated traits involving physiological adaptation, phytohormone metabolism, and drought-inducible genes. A novel QTL for red rot resistance was located within a 14.6 Mb region on sorghum chromosome 5, indicating minor QTLs on alternative copies in sugarcane. Chen et al. (2022) performed a GWAS on leaf angle in 216 core sugarcane parents and their derivatives using 4,027,298 high-quality SNP markers. Through a linear mixed model, they identified 4089 genes in the confidence interval of significant loci, with 3892 functionally annotated genes. This study reveals key genetic factors associated with leaf angle. Yang et al. (2018) identified one major-effect and two minor-effect QTLs for SOR resistance in a biparental population of 173 individuals. However, the exact positions of these QTLs are unavailable, making it unclear if they correspond to those found in this study. Additionally, comparing QTLs' R^2 values between their biparental mapping and the current association mapping is not feasible due to differences in allele frequencies.

Table 15.3 List of genome-wide association studies (GWAS) for dissecting important sugarcane traits

Traits	Sample size	No. of markers used	GWAS model	QTLs identified	References
Sugarcane orange rust	568 interspecific hybrids	183,842 SNPs	Single and multi-locus GWAS	*Oru*1, *Oru*2, *Oru*3, *Oru*4 *Oru*5 and *Oru*6	Dijoux et al. (2024)
Leaf scald disease	170 genotypes	100 K SNP array	GLM, MLM, MLMM, SUPER, BLINK	13 MTAs	Li et al. (2024a, b)
Yield-related traits.	159 genotypes	25,429 SNPs	GLM and MLM	140 MTAs	Zhang et al. (2023)
Fiber contents and sucrose contents	384 and 237 genotypes	260 SSR, 950 AFLP and 100 TRAP	GLM, MLM and FarmCPU	13 MTAs and 9 MTAs	Xiong et al. (2023)
Sugarcane mosaic virus	97 genotypes	70,888 SNPs	MLM	9 MTAs	Pimenta et al. (2023)
Sorghum mosaic virus	213 genotypes	SNPs	MLM	33 MTAs	Zamora (2022)
Leaf angle	216 genotypes	40,27,298 SNPs	MLM	4089 genes	Chen et al. (2022)
Yield-related traits	110 genotypes	1346 SNPs	GLM and MLM	34 and 8 MTAs	Senthilkumar et al. (2022)
Red rot	305 genotypes	58,028 SNPs	Mixed model	29 MTAs	O'Connell et al. (2022)
Yield-related traits and sucrose content	97 genotypes	6299 SNPs and 235 InDels	MLM	54 and 209 MTAs	Fickett et al. (2019)
Yield component traits	34 genotypes	100 SSR	FarmCPU	23 MTAs	Barreto et al. (2019)
Fiber composition	299 genotypes	65,000 SNPs and 9900 InDels	General model, additive model, 1-dom-alt model, 1-dom-ref model, diplo-general model, and diplo-additive model	217MTAs	Yang et al. (2020)

Note: *BLINK* Bayesian-information and Linkage-disequilibrium Iteratively Nested Keyway, *FarmCPU* Fixed and random model circulating probability unification, *GLM* Generalized linear model, *InDels* Insertions and deletions, *MLM* Mixed linear model, *MTAs* Marker trait associations, *SNPs* Single nucleotide polymorphisms, *SSRs* Simple sequence repeats

15.8 CRISPR-Cas9 Genome Editing in Sugarcane Breeding

Genome or gene editing encompasses advanced techniques that allow scientists to precisely modify genome sequences and regulate gene expression in targeted regions. These methods provide novel insights into functional genomics and have gained significant attention in agriculture due to their simplicity, precision, and ability to enhance crop traits. Genome editing enables the addition of beneficial traits or the removal of undesirable ones, offering new avenues for developing improved crop varieties. With the availability of fully sequenced and annotated plant genomes, genome editing innovations have advanced our understanding of gene regulatory functions, plant-pathogen interactions, and genetic mechanisms. Techniques such as zinc-finger nucleases (ZFNs) and transcription-activator-like effector nucleases (TALENs) use engineered proteins to guide DNA nucleases, while the CRISPR/Cas system employs sequence-specific nucleases directed by a single guide RNA (sgRNA). Due to its precision, simplicity, and cost-effectiveness, the CRISPR/Cas9 system has become the preferred tool in plant breeding.

CRISPR/Cas9 induces double-strand breaks (DSBs) in DNA, which are repaired through mechanisms classified into three types of site-directed nuclease (SDN) applications based on regulatory guidelines (Buchholzer and Frommer 2023). SDN1: DSBs are repaired via non-homologous end joining (NHEJ), which introduces random insertions or deletions of nucleotides. This often leads to frameshift mutations but does not involve the use of external DNA templates. SDN2: DSBs are repaired through homologous recombination (HR) using a synthetic DNA template. This allows precise editing, such as targeted nucleotide additions, deletions, or replacements. SDN3: Homologous recombination incorporates gene segments or entire genes at specific sites in the genome. Depending on the source and nature of the inserted genetic material, SDN3 edits may result in transgenic products.

These genome editing tools have been widely used over the past decade to improve crop adaptability and enhance agricultural productivity (Ndudzo et al. 2024). In sugarcane, this approach offers precision in modifying traits such as lignin content, stress resilience, and pathogen resistance. For instance, CRISPR/Cas9-mediated editing of the *SoLIM* gene significantly reduced lignin content in sugarcane stalks, enhancing its suitability as a feedstock for bioethanol production (Laksana et al. 2024). Additionally, CRISPR enables the development of virus-resistant sugarcane varieties through targeted genome manipulation and multiplex editing, effectively managing viral diseases (Surya Krishna et al. 2023). Advanced strategies, such as template-mediated homology-directed repair (HDR), have facilitated precise co-editing of multiple alleles, such as herbicide tolerance-conferring genes, demonstrating potential for targeted trait enhancement (Oz et al. 2021). Furthermore, sugarcane serves as a model for gene editing in other polyploid and aneuploid grasses, highlighting the versatility and adaptability of CRISPR tools such as Cas9, Cas12, and SpRY (Li and Iqbal 2024). These advancements underscore the transformative role of CRISPR/Cas technology in overcoming genomic complexities and accelerating sugarcane improvement.

15.9 Challenges for Deploying Genomics-Assisted Breeding

GAB shows promise for improving crop yields, resistance, and other agronomic traits, but applying it to sugarcane involves unique complexities. Unlike many crop genomes, the sugarcane genome is particularly complex. It is highly aneuploid, meaning it has an unbalanced number of chromosomes, and it is polyploid, meaning it contains multiple sets of each chromosome. This structure results in a genome with numerous chromosomal copies and an intricate mix of alleles, which poses significant challenges for precise genotyping. Furthermore, sugarcane's growth cycle is much longer than that of many other crops, spanning months to years. This extended cycle means sugarcane plants are exposed to various environmental conditions over time. Consequently, the environmental factors influencing a plant at one stage of its development might differ significantly from those at another stage. This variability complicates phenotyping, the process of measuring and evaluating plant traits, as sugarcane's traits can vary depending on the growth stage and external conditions, making it harder to identify stable, heritable traits.

GS models, commonly used in GAB, also present challenges for sugarcane. Most GS models were initially developed for seed-propagated crops (such as corn or wheat), where breeding strategies often emphasize additive effects, the contributions of individual genes to traits. These models tend to treat other genetic interactions, like dominance (interaction between alleles of a gene) and epistasis (interaction between different genes), as residual effects or noise. However, dominance and epistatic effects become more significant for clonally propagated crops like sugarcane, where plants are reproduced by cloning rather than seeds (Sandhu et al. 2022). This is because all alleles, including those with dominant or epistatic interactions, are preserved precisely in each new generation through clonal reproduction, meaning that these interactions influence plant traits across generations and are essential to accurate genomic predictions. Moreover, allele dosage variations, which refer to differences in the number of copies of each allele (due to the polyploid nature of sugarcane), add another layer of complexity to genomic prediction accuracy (Sandhu et al. 2022). These dosage variations can influence trait expression, but they're often not fully accounted for in standard GS models.

15.10 Conclusion

Sugarcane offers substantial potential as a source of sugar and lignocellulosic biomass for second-generation biofuels. Still, its complex genome presents challenges for fully optimizing traits related to both sugar and biofuel production. Progress in breeding, molecular genetics, and NGS has produced improved varieties, laying the foundation for accelerated crop improvement. Future breakthroughs will rely on deeper genomic insights, particularly from complete genome sequencing, to identify significant genomic loci for biomass optimization. Integrating GS with

traditional breeding approaches is critical to enhancing genetic gains in complex traits. Additionally, the precision and efficiency of the CRISPR-Cas9 system are expected to drive rapid, beneficial genetic advancements in crop plants. In parallel, advances in AI and ML are likely to transform agricultural planning and decision-making, enhancing product quality and adaptability. Together with advanced phenotyping, genotyping, and computational methods, these innovations will streamline sugarcane breeding, facilitating the development of high-yield varieties designed for sustainable production.

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Chapter 16
MicroRNAs: Small RNAs with a Big Role in Sugarcane Development and Breeding

Garima Pathak, Nidhi, and Ao-Mei Li

Abstract Short RNA molecules known as microRNAs (miRNAs) play a crucial function in regulating gene expression after transcription. They do not code for proteins. They are necessary for plants to develop and react to stress. miRNAs are crucial for regulating many physiological processes, including development, growth, and stress tolerance in sugarcane plants, a polyploid crop of great agricultural significance. Due to their capacity to regulate gene expression in reaction to both living and non-living factors, miRNAs show potential as valuable candidates for enhancing sugarcane through breeding initiatives. Recent studies have emphasized the significance of some miRNAs in regulating characteristics such as sucrose accumulation, biomass yield, and resistance to illnesses and environmental challenges. Specifically, particular miRNAs have been identified to selectively regulate genes that play a role in carbohydrate metabolism, thereby directly influencing the sugar content, which is a crucial characteristic in the process of breeding sugarcane. Others control genes that provide resistance to pathogens and unfavourable environmental circumstances, such as drought and salinity. Integrating miRNAs into sugarcane breeding programs is a new method for improving crop performance. Breeders can enhance sugarcane cultivars by finding and altering miRNAs linked to favourable characteristics, resulting in increased yield, better sugar content, and enhanced resistance to various pressures. Biotechnological methods, such as gene editing and miRNA-based genetic engineering, can be utilized to make exact adjustments in miRNA expression, resulting in specific enhancements in sugarcane. Although

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there is great potential, there are still obstacles to completely comprehending the intricate regulatory networks that involve miRNAs and their interactions with other genetic components. Future research should prioritize the investigation of these networks and the development of efficient ways for manipulating miRNAs. This is crucial for the successful incorporation of miRNAs into sugarcane breeding programs.

Keywords microRNAs · Sugarcane breeding · Gene expression regulation · Stress tolerance · Sucrose accumulation · Genetic engineering · miRNA manipulation · Crop improvement

16.1 Introduction

Saccharum ssp. belongs to the tribe *Andropogoneae,* subfamily *Panicoideae,* and family *Poaceae.* Polyploid perennial plants that use a C_4 photosynthetic pathway make up the majority of the *Andropogoneae* tribe (Clayton and Renvoize 1982). One of the biggest tribes in the Poaceae family, it is widely distributed across tropical and subtropical parts of the world (Clayton and Renvoize 1982; Sánchez-Ken and Clark 2010; Afghan et al. 2024). Sugarcane is one of the most photosynthetically efficient crops, significantly contributing to global sugar production. About 75% of the world's sugar is produced from sugarcane, a crop of major economic significance (Commodity Research Bureau 2015). It is also becoming more and more important in the generation of renewable energy. Sugarcane, which is grown in around 111 countries worldwide, provides 40% of the ethanol and 80% of the sugar used globally (Zhang et al. 2013; Verma et al. 2022a, b). According to Botha and Black (2000), sugarcane has a unique ability to accumulate sucrose, with the stalks containing concentrations of up to 50% of its dry weight. *Saccharum* spp. are the multi-species hybrids that are grown all over the world and are the result of conventional breeding techniques. The breeding operations were based on *Saccharum officinarum* because of its high sucrose concentration in the stem, although this cultivar is not very resistant to disease (Song et al. 2019; Singh et al. 2019). Due to its robustness and resilience to pests, *S. spontaneum* is an important species in the breeding program (Miranda et al. 2008). According to Swapna and Kumar (2017), this crop's notable polyploidy and genomic features point to a complex regulation system for commercially crucial traits, including sugar content, cane production, and tolerance to biotic and abiotic challenges (Verma et al. 2019, 2021a, b). Different types of RNAs are among the many genes and regulatory elements involved in these metabolic activities (Xu et al. 2022).

Through post-transcriptional and post-translational changes, the interaction between RNA and target sequences may control several activities. Many commercially critical sugarcane properties are predicted to be regulated by microRNAs

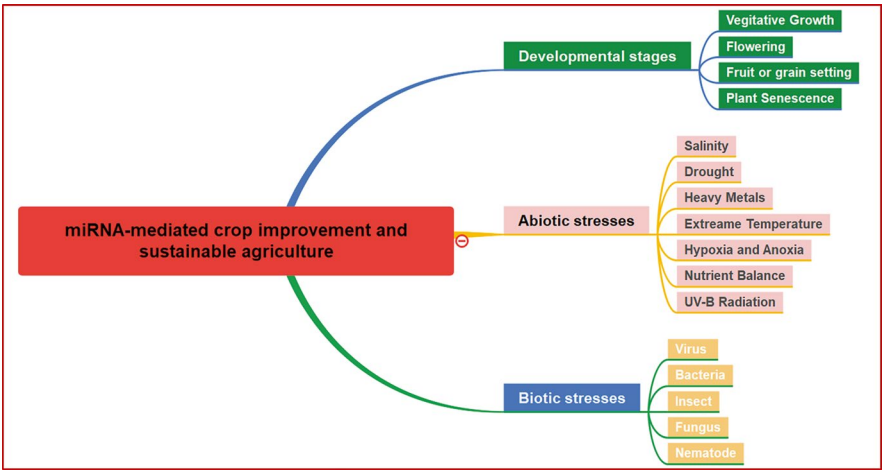

Fig. 16.1 A summary of miRNA-driven enhancements in crop production. Generally, miRNA and their target genes enhance plant growth at several developmental stages, increase biomass, and regulate various pathways

(Thiebaut et al. 2012; Ferreira et al. 2012; Srivastava 2013; Gentile et al. 2013, 2015; Lin et al. 2014). With the rising global demand for sugar and renewable energy (Li et al. 2024), it is essential to improve sugarcane yield and resilience using advanced breeding techniques. One class of tiny regulatory RNAs known as microRNAs (miRNAs) has been identified as having a major role in the development and breeding of sugarcane, as summarized in Fig. 16.1. This paper examines the existing knowledge of miRNAs in sugarcane and their prospective applications in enhancing crop performance.

16.2 Overview of MicroRNAs

The tiny regulatory RNAs known as microRNAs (miRNAs) are essential for many aspects of plant development (Nogueira et al. 2007; Rubio-Somoza et al. 2009; Chitwood et al. 2009), signal transduction, protein degradation, and reactions to biotic and abiotic stress (Shukla et al. 2008; Ruiz-Ferrer and Voinnet 2009; Li et al. 2023). MicroRNAs are generated by the successive processing of primordial miRNA transcripts (pri-miRNAs) by RNA polymerase II (Pol II). Prior to being processed into a stem-loop precursor (pre-miRNA) and then cleaved into an RNA duplex by the DICER-LIKE1 (DCL1) enzyme, pri-miRNAs often assume an erroneous fold-back structure (Zanca et al. 2010a, b). By slicing inside miRNA-mRNA base-pairing, miRNA-associated RNA-induced silencing complexes (RISCs) may promote translational repression, rapid exonucleolytic mRNA degradation, and/or mRNA cleavage when miRNA and its target RNA are partially or completely

base-paired (Voinnet 2009). Developmentally important transcription factors are the most common target genes of highly conserved miRNAs (Rhoades et al. 2002). However, lineage-specific miRNAs seem to control the expression of a wider range of genes, including those linked to post-translational modifications, stress response, and cellular metabolism (Zhao et al. 2010).

16.3 miRNAs in Sugarcane: Modulating Diverse Pathways

Numerous crops have been studied for their function in miRNA regulation of genes (Jones-Rhoades et al. 2006; Mallory and Bouche 2008; Axtell and Bowman 2008; Ding et al. 2013; Saikumar and Kumar 2014; Srivastava et al. 2015). In sugarcane, miRNAs are believed to regulate different characteristics significantly (Zanca et al. 2010a, b). There has been little research done on the discovery and expression analysis of mature miRNAs in this crop. The lack of a well-sequenced entire sugarcane genome and the crop's inherent complexity complicate miRNA investigations in sugarcane. Identification of miRNA precursors in EST datasets is made more difficult by their intrinsic instability (Gentile et al. 2015). A sequenced genome is necessary for the identification of novel miRNAs and the prediction of their targets; however, wet bench cloning and in silico sequence mining may help with this challenge in the early stages. Utilizing data from Sorghum, sugarcane's closest diploid cousin, for miRNA discovery and subsequent study in sugarcane has been made possible by the use of comparative genomics (Zanca et al. 2010a, b). The types of miRNAs that are present and their abundance in connection to certain properties have been revealed by the few studies on miRNA-based regulation in sugarcane. MiRNAs longer than 24 nucleotides were found in sugarcane genotype libraries that responded to salt and drought stress, while 21 nt miRNAs were more abundant in libraries that were resistant to drought stress. The selected target and the assessed feature determine how much each of these two miRNA types contributes to the regulation of a certain pathway. According to Thiebaut et al. (2012), 24 nt miRNA candidates were found to target the 60S acidic ribosomal protein RPP2B, which is linked to biotic stress tolerance, while 21 nt miRNAs identified the proposed miRNA target MADS2, a MADS-domain transcription factor involved in developmental regulation.

16.4 Drought Response and miRNAs in Sugarcane

For sugarcane, drought is the most studied aspect of miRNA-mediated regulation (Thiebaut et al. 2012; Ferreira et al. 2012; Srivastava 2013; Gentile et al. 2013, 2015; Lin et al. 2014). Six hundred twenty-three putative new mature miRNAs were found using computational analysis using sequences from four sugarcane genotypes subjected to biotic and abiotic stressors: four drought-tolerant and four

drought-sensitive (Thiebaut et al. 2012). Forty-four of them were identified as high-confidence miRNAs. Twenty of the 67 miRNAs that were specifically found in the water deficit experiment were also found in the susceptible and tolerant assays. Ferreira et al. (2012) found that seven of the 18 miRNA families were differently expressed during drought in sugarcane cultivars with variable levels of drought tolerance (Chen et al. 2020). The degree and duration of water stress caused variations in the differential expression. Lin et al. (2014) examined the expression profile of miRNAs in the leaves of the drought-resistant cultivar ROC22 and found 438 likely target genes in addition to 23 conserved and 34 new miRNAs. Eleven miRNAs were found to exhibit differential expression between the control and drought-exposed plants.

16.4.1 miRNAs Controlling Additional Sugarcane Stresses

Apart from drought response, miRNA-mediated regulation in sugarcane has also been studied in relation to salinity and disease responses. Compared to cultivars under light salt stress, Carnavale-Bottino et al. (2013) found 11 miRNAs with increased expression in *Saccharum* spp. cultivars under severe salt stress. The miRNAs linked to the occurrence of diseases in sugarcane as well as the gene sequences that act as targets for pathogen miRNAs have been investigated. Over 240 miRNAs in sugarcane affected with *Acidovoraxavenae* ssp. *Avenae* were found by Thiebaut et al. (2012). The miRNAs produced by the Sugarcane Streak Mosaic Virus (SCSMV) that impact the sugarcane genome were computationally predicted and empirically verified by Viswanathan et al. (2014), who also identified the possible gene targets of these miRNAs in sugarcane. The pathogen was found to have thirty putative miRNAs. In sugarcane, 19 target genes belonging to different gene families were discovered for the miRNA SCSMV miR16.

16.4.2 miRNAs and the Regulation of Biomass-Related Characteristics

Research on the metabolic pathways linked to lignin and the breakdown of cellulosic material in sugarcane has grown in importance as the crop becomes a feasible feedstock for cellulosic bioethanol. Certain biofuel crops were discovered to include microRNAs linked to development and stress response, as well as those linked to recalcitrance and bi-confinement (Trumbo et al. 2015; Liang et al. 2024). In sugarcane, miR156 and miR159 have been shown to be associated with abiotic stresses, bioconfinement, and recalcitrance. These researchers have found several other miRNAs linked to biomass enhancement in relevant biofuel crops, including miR164, miR166, miR167, miR172, miR398, miR414, miR444, miR477, miR528, miR531,

miR854, miR1535, miR1848, miR102, and miR2118. These miRNAs may also be important in sugarcane.

The amount of lignin in switchgrass decreases by 30% when miR156 is overexpressed. It is thought that the relative levels of miR156 and miR172 alter biomass content via influencing the transition from juvenile to adult stages. A protein involved in the metabolism of sucrose, Trehalose-6-Phosphate Synthase-1, is known to be downregulated by miR156. Phase transition, sugar synthesis, shoot development, and floral development have all been shown to be impacted by miR166, miR169, and miR139.

16.5 Sugar Metabolic Pathway and miRNAs in Sugarcane

Despite the importance of sugar accumulation and eventual sugar content as economic factors in sugarcane, no miRNAs involved in sugar metabolism have been discovered thus far.MiR156 and miR139 regulate the enzymes sucrose synthase and trehalose-6-phosphate in switchgrass (*Panicum virgatum* L., Xie et al. 2014). Differentially expressed miRNAs, including miR169, were found in grain and sweet sorghum by Calvino et al. (2011) and Yu et al. (2015), who hypothesized that this miRNA could be connected to the accumulation of sugar in the crop.

16.5.1 Applications of MicroRNAs in Sugarcane Breeding

16.5.1.1 Enhancing Sugarcane Traits

- The manipulation of miRNAs offers a novel strategy to improve desirable characteristics in sugarcane, including enhanced biomass, stress tolerance, and disease resistance. Genetic engineering techniques can either overexpress or inhibit specific miRNAs in sugarcane.

16.5.1.2 Genetic Engineering Approaches

- *Overexpression*: To increase sugar yield, it is possible to postpone blooming and lengthen the vegetative period by targeting the overexpression of microRNAs, such as miR156.
- *Gene Silencing*: Silencing miRNAs that negatively control characteristics, such as stress tolerance or disease resistance is possible by RNA interference (RNAi).
- *Marker Development for Breeding Programs*: One way to improve breeding efficiency is to find and use molecular markers related to miRNA. With the help of these markers, marker-assisted selection (MAS) algorithms can be implemented, allowing breeders to choose traits associated with miRNAs.

16.6 Conclusion and Future Perspectives

There are still several obstacles to overcome, even though miRNAs show great promise in sugarcane breeding and improvement. Functional study and miRNA characterization are made more challenging by the intricacy of sugarcane's polyploid genome. Furthermore, it is vital to create effective delivery mechanisms for practical applications of genetic engineering procedures based on microRNAs. MicroRNAs are crucial in regulating sugarcane development and possess substantial potential for enhancing desirable traits via breeding efforts. Advances in miRNA function research imply that adding miRNA-based techniques into breeding programs might aid in the production of hardy and high-yielding sugarcane variants. Future research must address existing challenges and fully utilize the potential of miRNAs in sugarcane breeding.

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Chapter 17
Sugarcane Genomics and Transgenic Breeding

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Abstract The public availability of the complete genetic blueprint of sugarcane has significantly accelerated research in structural, functional, and applied sugarcane genomics. Current genome research initiatives are expanding beyond single-species representation to encompass the sequencing of multiple closely related genomes within a species. These endeavors leverage next-generation sequencing technologies, enabling more precise, efficient, and cost-effective genome sequencing, resequencing, and assembly at unprecedented depths. The sugarcane genomic resources developed thus far are invaluable for investigating its unique biology and enhancing the crop's quality and productivity, particularly given its importance as a first-generation biofuel crop. Sugarcane cultivars have substantially benefited from biotechnological advancements, especially transgenic breeding techniques that complement conventional breeding approaches. While recent decades have focused on enhancing sugarcane breeding strategies, future research directions should prioritize the utilization of the extensive sugarcane genomic resources and advancements achieved through transgenic breeding methodologies.

Keywords Sugarcane genomics · Sequencing · Biofuel · Conventional breeding · Transgenic

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17.1 Introduction

Saccharum spp., commonly referred to as sugarcane, is cultivated in warm, tropical, or subtropical climatic conditions. This crop has a rich history spanning over two millennia and holds significant importance in global agriculture, contributing approximately 80% of the world's sugar supply and 40% of ethanol production (Meng et al. 2020; Verma et al. 2021a, b, 2024a, b). Sugarcane, a member of the Poaceae family, was among the earliest plants to stimulate human technological advancement (Goldstein and Mintz 2015). This crop is cultivated across approximately 80 countries, covering an area of 26.89 million hectares and yielding a global production of 1922.06 million tonnes (FAOSTATS-2023). Sugarcane, a highly intricate crop, exhibits significant polyploidy and aneuploidy, resulting in multiple allelic representations of each gene within its genome. Sugarcane is commercially grown vegetatively from the axillary buds of stem segments. Its typical harvest cycle ranges from 12 to 24 months post-planting, with subsequent harvests derived from regrowth of the stubble, commonly referred to as "ratoon" crops (Song et al. 2019; Singh et al. 2021; Afghan et al. 2024).

However, micropropagation via tissue culture is a more suitable method than vegetative bud propagation and is used widely in some nations to develop new varieties. Conventional taxonomy classifies the genus *Saccharum* to include six species: *S. officinarum*, *S. sinense*, *S. barberi*, *S. edule*, *S. robustum*, and *S. spontaneum* (Fig. 17.1). The global cane sugar industry relies only on interspecific hybrid plants of *Saccharum* spp., which have mostly been supplanted by the noble canes, *Saccharum officinarum*. Approximately 70–80% of the genomic content of contemporary sugarcane cultivars originates from *S. officinarum,* whereas 10–20% is derived from *S. spontaneum*. Modern sugarcane cultivars are aneuploid plants resulting from the hybridization of *S. officinarum* and *S. spontaneum*, followed by many backcrosses to *S. officinarum* (Amalraj and Balasundaram 2006; Malviya et al. 2020; Huang et al. 2022, 2024). This complex hybridization process represents a milestone in sugarcane breeding, enhancing vigor, robustness, tillering, disease resistance, and environmental adaptation while increasing the genomic complexity beyond their progenitors.

Transgenic technology provides an alternative approach to plant breeding and must be integrated with traditional breeding to develop new varieties and products. In conventional breeding, the breeder finds favorable recombination among a wide segregating population. Still, in transgenic breeding, the breeder identifies a specific phenotypic trait and then introgresses through the transgenic event into a diverse array of favorable genetic backgrounds. Sugarcane produces over 80% of worldwide sugar production, and sugar beetroot produces the remaining 20% (Verma et al. 2019a, b; Babu and Adeyeye 2024).

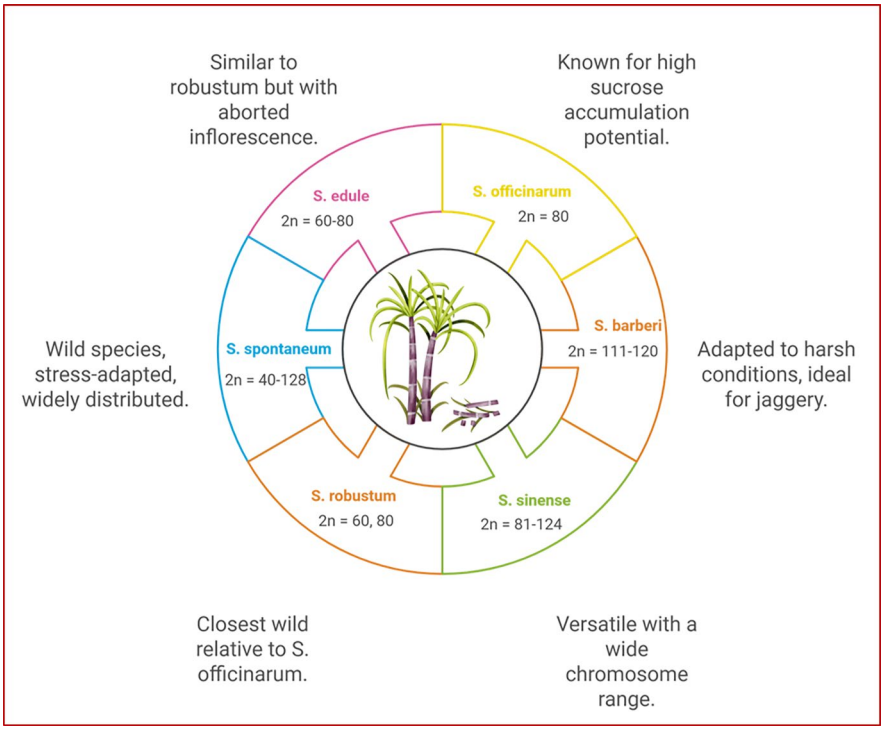

Fig. 17.1 Diversity of the genomic constitution of *Saccharum* species and their distinct characteristics. (Created by Napkin AI: https://www.napkin.ai/)

Consequently, within the framework of global commerce and economics, sugarcane persists as a significant food commodity and a key industrial and bioenergy resource (Mirajkar et al. 2017; Singh et al. 2020a, b; Chen et al. 2020). Conventional sugarcane breeding is prohibitively expensive, highly labor-intensive, and protracted (requiring 10–15 years to develop a new elite variety) due to genetic complexity, a limited genetic basis, poor breeding progress, and vulnerability to significant diseases and pests (Oliveira et al. 2022; Guo et al. 2023; Liang et al. 2024). Nevertheless, the economic significance of sugarcane, investigations into its genome, and establishment of a reference genome or pan-genome have been delayed owing to its elevated ploidy levels and intricate genomic structure. Despite the considerable hurdles, modern biotechnology methods, including genomics and transgenic breeding, offer a potential pathway for improving sugarcane resilience and output (Fig. 17.1). Nonetheless, regulatory obstacles and societal acceptability of genetically modified organisms remain significant issues that may hinder advancement in this field (Surya Krishna et al. 2023).

17.2 Sugarcane Genomics

17.2.1 Sugarcane Genome Complexity

The modern cultivars of sugarcane originated from interspecific hybridization between *S. officinarum* (2n = 8x = 80; x = 10) and *S. spontaneum* (2n = 5x–16x = 40–128; x = 8). *S. spontaneum* exhibits the highest genetic diversity in the *Saccharum* genus, with (2n = 40–128). Chromosome multiples of eight are common, suggesting a basic chromosome number of x = 8, though the ploidies and chromosome numbers of non-multiples in *S. spontaneum* accessions remain unclear (Ming et al. 2006). The indigenous hybrid breeding strategy results in a contemporary sugarcane hybrid originating from both ancestral parents. However, it introduces genome complexity due to the unequal genetic contributions from each parent during the hybridization process, the non-directional loss of chromosomes during transmission, and the formation of recombinant (Rec) chromosomes from both progenitors. Sugarcane noted for its significant aneuploidy, polyploidy, and heterozygosity, possesses a large genome size, which poses challenges in the creation of genomic and transcriptome resources aimed at crop improvement (Meena et al. 2022). For many years, the genomics of sugarcane has lagged behind that of other grass species, including rice, wheat, barley, and sorghum, due to its highly complex and polyploid genome (Singh et al. 2021a, b; Guo et al. 2021, 2022). Loss of chromosomes in a non-directional manner during transmission, along with the appearance of recombinant (Rec) chromosomes from both parent sources. The genetic diversity across several *Saccharum* genotypes has been characterized in organelle and nuclear genomes (D'Hont et al. 1995; Sobral et al. 1994).

A diverse set of DNA markers has been used to evaluate the genetic diversity within the nuclear genome of *Saccharum* species. *Saccharum* is well recognized for its polyploidy, with octopolyploids appearing most often in *S. spontaneum*, *S. officinarum*, and *S. robustum* (Irvine 1999; Wu et al. 2022a, b). The sugarcane genome is around 10 GB, making it more significant than any other member of the Poaceae family (Aitken et al. 2016; Xu et al. 2022). Thus, the sugarcane genome is a key component that provides insights into several fascinating characteristics of sugarcane. Recent genomic advancements offer a significant chance to integrate diverse alleles into breeding programs by gene mining from wild relatives (Abberton et al. 2016; Anas et al. 2021a, b). Several studies have examined the genetic diversity of sugarcane cultivars and progenitor species using various molecular markers. Less variation was observed in the mitochondrial genomes of *S. officinarum*, *S. barberi*, *S. sinense*, and *S. edule* compared to *S. spontaneum* (an allopatric wild species), which exhibited unique RFLP patterns relative to the other *Saccharum* spp. (D'Hont et al. 1995).

17.2.2 Advances in Sugarcane Genomics

Advancements in DNA sequencing technologies and genome assembly strategies are enhancing the accessibility of the sugarcane genome. Next-generation sequencing (NGS) methods progressively reveal the intricate nature of the sugarcane genome, and, likely, sugarcane will soon serve as a model for investigating other complex, polyploid genomes. Current sequencing efforts frequently produce chimeric assemblies because of the high nucleotide identity among subgenomes, complicating their differentiation (Shearman et al. 2022). The assembly of the sugarcane genome necessitates the use of long-read sequencing technologies, like PacBio, in conjunction with chromatin conformation capture to accurately scaffold the genome for the assembly of the highly polyploid sugarcane genome (Manimekalai et al. 2020; Shearman et al. 2022; Bao et al. 2024). The process of genome sequencing, along with the development of genomic resources like the SUEST (sugarcane expressed sequenced tag) database, genetic maps, transcriptomes, and bacterial artificial chromosome (BAC) libraries (Souza et al. 2011; Malviya et al. 2021, 2022), will contribute significantly to our comprehension of the evolution of sugarcane's intricate genome and polyploid structure.

The advancements in these technologies have greatly improved our understanding of the intricate genome of sugarcane, thereby aiding in the enhancement of breeding and genetic research. Next-generation sequencing has transformed the field of genomics by facilitating high-throughput sequencing, which simultaneously permits the swift analysis of millions of DNA fragments (Sharma et al. 2021; Satam et al. 2023). In sugarcane, next-generation sequencing plays a crucial role in the identification of single nucleotide polymorphisms (SNPs) and the development of genotyping platforms, including SNP microarrays and genotyping by sequencing (GBS) (Manimekalai et al. 2020). GBS has shown significant efficacy in identifying SNPs on a large scale within various sugarcane germplasm, thereby improving genomic selection and marker-assisted breeding (Manimekalai et al. 2020). The PacBio Isoform sequencing has been employed to investigate the intricate transcriptome of sugarcane, providing valuable insights into gene expression and isoform diversity (Hoang et al. 2017). Studies suggest that although GBS provides a greater read depth, RADSeq has proven more effective in clarifying genetic diversity and population structure in sugarcane breeding programs (Riascos et al. 2016). These technologies facilitate a thorough methodology in sugarcane genomics, improving breeding strategies and trait associations.

The complex hybridization of modern cultivars like R570 presents challenges in identifying distinct genetic sequences, mainly due to significant recombination (Healey et al. 2024). The recent advancements in genome assemblies of sugarcane hold considerable importance for breeding programs focused on improving traits like yield, disease resistance, and adaptability in sugarcane (Singh et al. 2021; Song et al. 2021, 2022). These assemblies offer an in-depth insight into the complex structure of polyploid genomes, allowing breeders to utilize genetic diversity and enhance their selection methods. The chromosomal scale assemblies of hybrid

sugarcane cultivars, including ZZ1, showed 10.4 Gb of genomic sequences containing 68,509 annotated genes, emphasizing gene families corresponding with sugar accumulation and disease resistance (Bao et al. 2024). The polyploidy genome assembly of the R570 cultivar, which covers 8.7 billion bases, provides valuable insights into its genetic architecture and identifies potential molecular targets for breeding (Healey et al. 2024). The SP80-3280 assembly revealed 373,869 putative genes, highlighting the functional diversity essential for enhancing biomass and sugar production (Souza et al. 2019). The comprehensive genomic data facilitates a more accurate selection of characteristics, including disease resistance and sugar yield, which have reached a standstill in conventional breeding (Singh et al. 2022; Krishna et al. 2022; Healey et al. 2024). The assemblies enable the recognition of advantageous alleles and gene families, supporting the introgression of traits from wild relatives such as *Erianthus fulvus* (Kui et al. 2023).

17.2.3 Comparative Genomics

Comparative genomics in sugarcane (*Saccharum* spp.) constitutes an essential approach for deciphering its complex genome and improving breeding methodologies. Recent studies have provided the perspective on the genetic architecture, evolutionary dynamics, and functional conservation of key gene families, which are crucial for enhancing sugarcane traits. Flow cytometry has been employed to evaluate genome size variations among various sugarcane genotypes, emphasizing the influence of aneuploidy on genetic variability (García et al. 2024). Through comparative genomics with sorghum, conserved genomic regions linked to bioenergy traits have been identified, aiding in focused breeding initiatives (Joshi et al. 2022; Federico et al. 2022). Comparative genomics provides important insights into the genetic structure of sugarcane; however, there are significant challenges in applying these findings to practical breeding strategies, mainly due to the crop's intricate polyploid characteristics.

17.2.4 Genomic Resources and Tools

The advancement of molecular markers and bioinformatics tools has dramatically greatly enhanced sugarcane breeding and genetic analysis. Based on expressed sequence tags (ESTs), SSRs play a vital role in genetic characterization and breeding, with a total of 4960 SSRs identified from 4120 unigenes. High-throughput sequencing has facilitated the development of SNP markers, which are crucial for determining parentage and protecting intellectual property in sugarcane (Saleem et al. 2022; Sharma et al. 2022; Aitken 2022). Molecular markers, such as SSRs and SNPs, evaluate genetic diversity among sugarcane varieties, uncovering unique genetic patterns and relationships (Hasan et al. 2023). Recent advancements

encompass a comprehensive monoploid sugarcane genome, facilitating the identification of genes associated with sugar accumulation (Saleem et al. 2022; Deng et al. 2023; Li et al. 2023a, b). Bioinformatics tools enable the examination of genetic data, improving breeding strategies and improving genomic research (Diniz et al. 2019). The combination of QTL mapping and GWAS in sugarcane enhancement has notably propelled the development of high-yield and resilient varieties. The utilization of genomic methods allows for the discovery of genetic markers linked to important traits, thereby enhancing the efficiency of selection processes in breeding initiatives.

QTL mapping has revealed a variety of loci linked to essential characteristics, including yield, fiber, and sucrose content. A study involving 159 sugarcane genotypes identified 36 QTLs associated with brix and 104 QTLs related to other yield traits (Chen et al. 2023; Zhang et al. 2023a, b). Moreover, 26 QTLs related to tillering and ratooning were identified, showing significant heritability that suggests their potential for selection (Singh et al. 2023; Wang et al. 2023; Bhatt et al. 2024). GWAS has played a crucial role in identifying specific SNP markers linked to traits such as fiber and sucrose content, thereby improving the accuracy of breeding initiatives (Xiong et al. 2023). A recent study identified 13 markers related to fiber and nine associated with sucrose, highlighting the efficacy of GWAS in trait association (Xiong et al. 2023; Li et al. 2024a, b). Additionally, GWAS has been employed to enhance disease resistance by identifying SNPs associated with red rot resistance, thereby improving selection accuracy in breeding programs (O'Connell et al. 2022; Chen et al. 2024). Integrating QTL mapping and GWAS with genomic selection enhances the breeding process by facilitating the selection of superior genotypes through genetic markers, moving beyond reliance on phenotypic evaluation alone (Tripathy 2022). This method decreases the time and expenses linked to conventional breeding and addresses challenges, such as linkage drag, thereby improving the overall effectiveness of sugarcane enhancement initiatives (Tripathy 2022).

17.3 Functional Genomics in Sugarcane

17.3.1 Transcriptomics

Transcriptomic studies in sugarcane have drastically improved the comprehension of its genetic responses to different stresses, especially drought and disease resistance. The study relies on comparative analyses to identify differentially expressed genes (DEGs) and their related pathways, offering valuable insights into the molecular mechanisms that drive these traits. RNA sequencing (RNA-seq) plays a crucial role in analyzing gene expression throughout various developmental stages, offering valuable insights into the dynamic alterations in the transcriptome (Tian et al. 2023; Horta et al. 2023). By providing a comprehensive view of the transcriptome, RNA-seq enables the identification of gene expression and RNA stability changes,

the detection of extensive transcriptome alterations during cellular stress, and the revealing of mechanisms like stress granule-dependent RNA decay (Solanki et al. 2023; Dar et al. 2024). This method achieves single-molecule resolution, allowing for the identification of variations in RNA length and their relationship with translation and ribosome occupancy during stress conditions (Dar et al. 2024). Stress-induced RNA decay has been demonstrated to rely on particular pathways, including XRN1, underscoring the complex nature of RNA metabolism under stress conditions (Dar et al. 2024). It is possible to model gene interactions and co-expression networks by employing bioinformatics tools, thereby enhancing the understanding of gene regulation during development (Malviya et al. 2024; Horta et al. 2023).

Comparative transcriptomic analyses showed substantial differences in gene expression between drought-resistant and susceptible sugarcane cultivars, particularly highlighting the pathways associated with starch and sucrose metabolism, photosynthesis, and stress signaling (Bhatt et al. 2023a, b; Zheng et al. 2023; Li et al. 2023a, b). The shoot-borne root (SBR) system in *Erianthus* showed a remarkable drought-adaptive architecture, characterized by the up-regulation of genes associated with stress signaling and osmoprotection contrast to commercial hybrids, which exhibited root cell damage when subjected to drought conditions (Valarmathi et al. 2023). A study on a high sucrose mutant clone identified key genes regulating sucrose metabolism, including Sucrose Phosphate Synthase (SPS), which was significantly upregulated, indicating its essential role in promoting sucrose accumulation (Khan et al. 2023a, b). The transcriptomic profiling of sugarcane genotypes in response to smut disease has unveiled a dynamic gene co-expression network and identified the hub genes associated with resistance mechanisms, particularly those involved in stress-related metabolic pathways (Wu et al. 2022a, b).

17.3.2 Proteomics and Metabolomics

Integrating proteomic and metabolomic methods in sugarcane studies has dramatically enhanced the comprehension of sucrose metabolism and plant defense mechanisms. Applying these omics technologies offers an in-depth perspective on the molecular interactions and biochemical pathways that play a role in sugarcane's interaction with environmental stresses and pathogen challenges. Proteomics has revealed more than 500 differentially abundant proteins (DAPs) related to sugar metabolism, highlighting essential enzymes that play a role in sucrose synthesis (Li et al. 2022). Proteins like glucose-6-phosphate isomerase and malate dehydrogenase are identified as crucial in the regulation of sucrose accumulation (Li et al. 2022). Metabolomic studies have identified unique metabolic profiles in sugarcane genotypes, especially in response to pathogens such as *Xanthomonas albilineans* (Bini et al. 2023). Genotypes that show resistance exhibited elevated concentrations of defence-related metabolites, including salicylic acid and phenolics, which play a vital role in the fight against leaf scald disease (Kumari et al. 2022, 2023; Bini et al. 2023). Recent studies emphasize the importance of particular metabolites, gene

expression, and endophytic interactions in improving sugarcane's resistance to diseases and environmental stresses. Metabolites, including citric acid, malic acid, and glutamate, are essential in the defense mechanisms of sugarcane against pathogens such as *Fusarium verticillioides* and *Sporisorium scitamineum* (Joshi et al. 2023; Xiao et al. 2024; Wang et al. 2020). Endophytic fungi play a significant role in disease resistance by outcompeting pathogenic fungi and improving metabolic functions in resistant sugarcane cultivars (Jagadesh et al. 2024; Xu et al. 2024; Xiao et al. 2024). Integrating proteomics and metabolomics provides a comprehensive insight into sugarcane physiology, discovering complex relationships among genes, proteins, and metabolites (Khan et al. 2023a, b; Ali et al. 2023).

17.4 Transgenic Breeding in Sugarcane

17.4.1 Introduction to Transgenic Breeding

Transgenic breeding facilitates the accurate transfer of one or more gene stacks from unrelated plant species or various organisms. Transgenic breeding techniques have notably enhanced sugarcane, especially via various approaches, including genetic engineering, genome editing, and germ-line gene transfer. Genetic engineering, often referred to as genetic modification, is a technique that facilitates the introduction of new traits into plants through various methods, such as *Agrobacterium*-mediated transformation, biolistics (or particle bombardment), electroporation, virus-mediated transformation, and PEG-based transformation, which is also known as protoplast-based transformation. The progress in genetic engineering has led to the creation of transgenic sugarcane varieties that display significant traits, such as resistance to herbicides, diseases, and pests, along with tolerance to abiotic stresses. Several investigations have demonstrated their outstanding performance in greenhouse settings and field trials.

The genome editing technique comprises the genetic modification of a crop, where a gene or a segment of DNA is either deleted, inserted, or substituted within the plant genome through the application of nuclease methods such as zinc-finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs), and meganucleases. A further technique is referred to as clustered regularly interspaced short palindromic repeats, commonly known as CRISPR/Cas9. These technologies improve characteristics essential for sucrose content, biomass yield, and biofuel production while tackling biotic and abiotic stresses. The first transgenic sugarcane plant was developed by Bower and Birch in 1992, who outlined a straightforward and effective method of microprojectile bombardment of embryogenic callus, resulting in transgenic plants featuring selectable genes. The CRISPR/Cas9 system has become a pivotal technology for crop advancement, facilitating precise genome editing through the generation of double-strand breaks at specific DNA locations, which enables targeted modifications (Ahmad and Ming 2024; Prado et al. 2024).

The elements of the CRISPR genetic system can be introduced into the target plant's genome through different methods, including DNA, mRNA (in vitro transcripts or IVT), and proteins (Eid et al. 2021). The *Agrobacterium*-mediated transformation method utilizes *Agrobacterium tumefaciens* for the transfer of genetic material into sugarcane cells, enabling the stable integration of desired traits (Laksana et al. 2024; Surya Krishna et al. 2023). RNA interference (RNAi) serves as a technique to silence particular genes, offering a means to manage unwanted traits or improve advantageous ones (Ahmad and Ming 2024). *Agrobacterium*-mediated transformation, Gene-gun method, CRISPR-Cas9 method are some of the techniques that widely used and have a significant potential for enhancing sugarcane (Fig. 17.2). This biotechnological approach presents a promising alternative to conventional breeding methods, which tend to be labor-intensive and time consuming because of the complex polyploidy genome of the crop.

17.4.2 Transgenic Traits in Sugarcane

Transgenic sugarcane varieties have shown significant advancements in traits such as drought tolerance, herbicide resistance, etc., enhancing their agricultural viability (Fig. 17.3). These innovations stem from various genetic modifications that address the challenges faced by conventional breeding methods.

17.4.2.1 Drought and Chilling Tolerance

Drought and chilling stresses significantly impact crop yield, growth, and development, leading to physical damages, physiochemical disruptions, and molecular alterations (Pandey et al. 2021; Verma et al. 2020a, b, 2021c, d), particularly in tropical crops such as sugarcane (Hussain et al. 2018; Verma et al. 2021e, f). The increased expression of sensitive (S) genes contributes to increasing plant drought susceptibility through hormonal imbalance, reduced antioxidant activity, and the production of reactive oxygen species (ROS). The overexpression of TERF1 in sugarcane via *Agrobacterium*-mediated transformation led to enhanced drought tolerance (Verma et al. 2020c, d, 2024c). This was achieved through the increased accumulation of osmoprotectants and reduced reactive oxygen species and malondialdehyde content. These effects may be attributed to the activation of stress-related gene expression by TERF1 in response to stress (Rahman et al. 2021).

The gene responsible for the enzyme isopentenyl transferase (ipt), regulated by the cold-inducible gene promoter AtCOR15a, was introduced into sugarcane (*Saccharum* spp.) cv. RB855536 through biolistic transformation to improve cold tolerance in sugarcane (Belintani et al. 2012). Overexpressing the SoTUA gene enhances transgenic sugarcane's cold tolerance (Chen et al. 2021). The expansion gene (*EaEXPA1*) from *Erianthus arundinaceus,* a wild relative of sugarcane, was overexpressed in the commercial sugarcane variety Co 86032 via

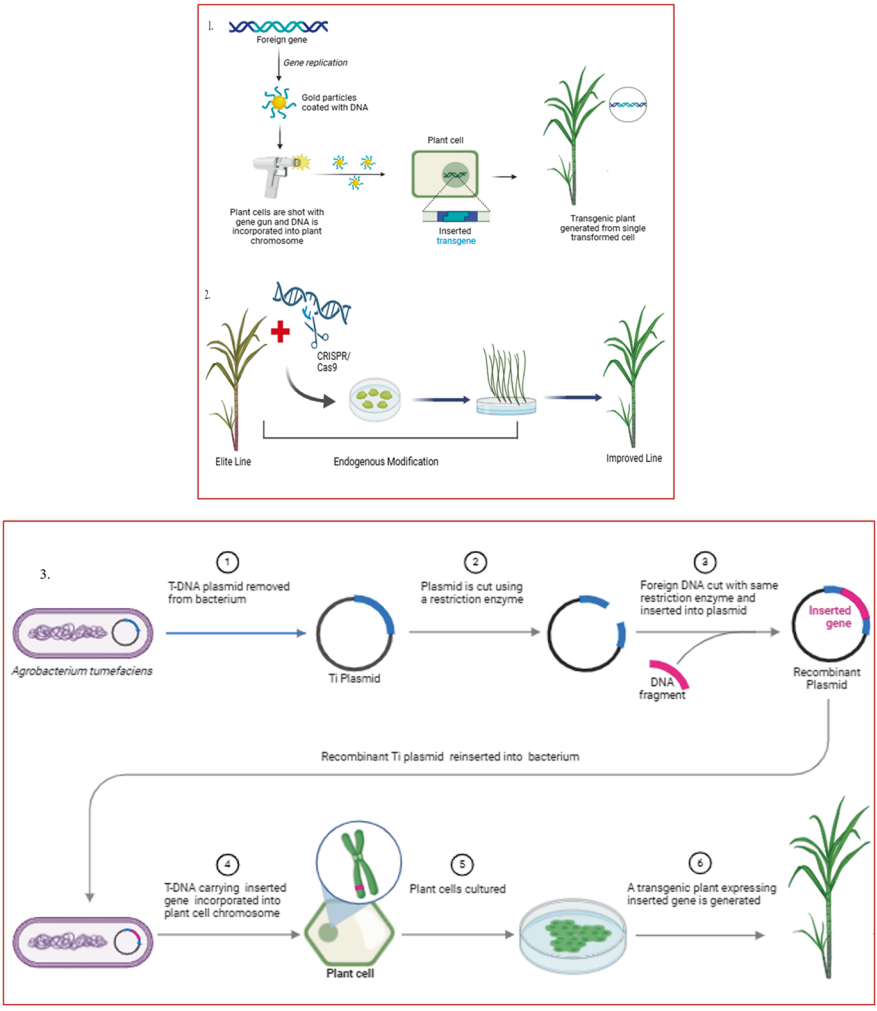

Fig. 17.2 Some advanced gene manipulation techniques in transgenic breeding (1) Gene gun method, (2) CRISPR/Cas9 method, and (3) *Agrobacterium*-mediated transformation. (Image created with BioRender.com)

Agrobacterium-mediated transformation. It indicates that the overexpression of EaEXPA1 in sugarcane enhances drought tolerance (Verma et al. 2020e, f; Narayan et al. 2021). The incorporation of the Arabidopsis vacuolar H+-pyro-phosphatase (H+-PPase) (AVP1) transgene into sugarcane plants via gene gun techniques has shown that the overexpression of AVP1 enhances drought tolerance in the plants (Raza et al. 2016). Transgenic sugarcane overexpressing the AtDREB2A CA gene has shown enhanced drought tolerance and increased sucrose accumulation in water-limited environments (de Souza et al. 2020; Verma et al. 2022a, b, 2023a,

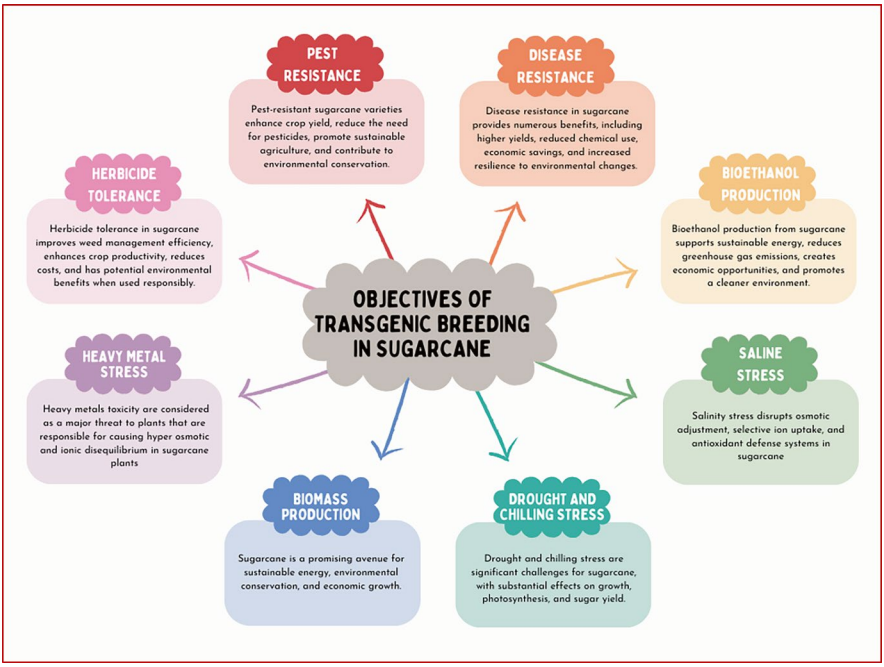

Fig. 17.3 Significance and goals of transgenic breeding in sugarcane. Image created with https://www.canva.com/

2024d). A cysteine protease (OVERLY TOLERANT TO SALT-1; OTS1) from *Arabidopsis thaliana* was genetically transformed using particle bombardment. This transformation with the AtOTS1 gene exhibited enhanced drought tolerance (Masoabi et al. 2023).

17.4.2.2 Heavy Metal and Salinity Stress

Intense exposure to salt and heavy metals can lead to the overproduction of reactive oxygen species in plants, potentially harming essential organic molecules such as DNA, proteins, and lipids. Both salt and heavy metal stress can adversely affect plant growth and metabolic processes. The GPCR-like protein (ShGPCR1) derived from sugarcane and energy cane (*Saccharum* spp. hybrids) is associated with enhanced tolerance to various abiotic stresses, particularly salinity tolerance (Ramasamy et al. 2021). The CRISPR/Cas9 technology has effectively enhanced the expression of the ScGluD2 gene, contributing to resistance against heavy metal and saline stress in sugarcane (Singh et al. 2020a, b). The assessment of overexpressing EaGly III for salinity stress tolerance at 100 mM and 200 mM NaCl revealed that transgenic sugarcane events overexpressing EaGly III demonstrated significant performance and enhanced salinity stress tolerance (Mohanan et al.

2021). The impact of salt stress on sugarcane plants that have been genetically modified with the *Vigna aconitifolia* P5CS gene, which encodes Δ1-pyrroline-5-carboxylate synthetase, regulated by a stress-induced promoter AIPC (ABA-inducible promoter complex). The response is evaluated, indicating a role in preventing oxidative damage in transgenic sugarcane subjected to salt stress (Guerzoni et al. 2014). The genetic engineering of sugarcane utilizing the *Oryza sativa* L. H+-PPase gene (OVP1) aims to enhance salt tolerance through tissue culture and biolistic transformation. The results demonstrate that the OVP1 gene was successfully expressed in the transgenic sugarcane lines (Ullah et al. 2022).

17.4.2.3 Herbicide Resistance

The development of herbicide resistance in transgenic sugarcane breeding represents notable progress in agricultural biotechnology, allowing for enhanced resilience to certain herbicides while preserving agronomic performance and crop yield. The initial effort to incorporate herbicide resistance into sugarcane was undertaken by Chowdhury and Vasil in 1992. They achieved this by transforming suspension culture cells through microprojectile bombardment and electroporating protoplasts with a plasmid that carried the bacterial gene bar, which encodes phosphinotrycin acetyltransferase. This gene serves as a selectable marker that imparts resistance to the herbicide Basta R, also known as glufosinate ammonium. The repair of DNA double-strand breaks induced by the programmable nuclease CRISPR/Cas9 utilized a template-mediated approach, resulting in multi-allelic precision nucleotide substitutions in the ALS gene of sugarcane. This was confirmed through Sanger sequencing, which demonstrated the conferred resistance to the herbicide nicosulfuron (Oz et al. 2021). The Bt insecticidal gene Cry1Ab, the glyphosate-tolerant gene EPSPS, and the selection marker gene PMI were combined into a single transferred DNA fragment and introduced into sugarcane through *Agrobacterium*-mediated transformation. The resulting transgenic lines exhibited robust insect resistance and glyphosate tolerance in both laboratory and field conditions (Wang et al. 2017).

Utilization of an effective *Agrobacterium*-mediated transformation system with the herbicide-resistant CP4-EPSPS gene as a selection marker was demonstrated, which can be used for future purposes due to the low cost of glyphosate and efficiency of this system (Wang et al. 2024). Despite the promising advancements in transgenic sugarcane, concerns regarding the long-term ecological impacts and potential resistance development in weeds remain significant.

17.4.2.4 Disease Resistance Genes

Transgenic sugarcane significantly improves disease resistance to various pathogens, such as fungi and viruses. The overexpression of the rice BROAD-SPECTRUM RESISTANCE 1 (BSR1) gene in sugarcane has demonstrated efficacy against both fungal and bacterial diseases, including sugarcane smut, utilizing a helium-driven

biolistic device that offers a simple approach for improving disease resistance in diverse crops (Maeda et al. 2023). The PAC1 gene from *Schizosaccharomyces pombe* was integrated into sugarcane using the *Agrobacterium*-mediated transformation method, developing virus-resistant varieties. These varieties showed milder symptoms and lower viral loads when challenged with the sugarcane streak mosaic virus (Wang et al. 2022). Significant contributions to lignin biosynthesis and enhanced resistance to the fungal pathogen *Sporisorium scitamineum*, which causes sugarcane smut, have been identified in four sugarcane varieties. The overexpression of these genes has shown improved resistance to multiple fungal pathogens, highlighting their potential application in breeding programs (Li et al. 2024a, b). Transgenic sugarcane was created through RNA interference aimed at the SCMVCp gene, which enhanced resistance against the sugarcane mosaic virus. The constructs driven by the Ubi promoter resulted in 82.35% resistant lines, showcasing the effectiveness of RNAi in improving disease resistance in sugarcane (Widyaningrum et al. 2021). Transgenic sugarcane incorporating the SUGARWIN1 and SUGARWIN2 genes has shown improved resistance to *Colletotrichum falcatum*, the pathogen responsible for red rot (Parvaiz et al. 2021). This genetic modification notably limited fungal growth, presenting a promising strategy for creating disease-resistant sugarcane varieties.

17.4.2.5 Pest Resistance

Transgenic sugarcane significantly improves pest resistance, especially concerning the sugarcane borer and other major pests. *Bacillus thuringiensis* (Bt) encodes crystalline proteins (Cry) that exhibit insecticidal activity. These Bt genes/proteins were introduced into embryogenic sugarcane calli through microprojectile bombardment. The resulting transgenic sugarcane lines, which show a higher level of Bt transcript expression, demonstrate increased resistance to the sugarcane borer *Diatraea saccharalis* (Budeguer et al. 2024). Transgenic sugarcane that expresses the *Bacillus thuringiensis* (Bt) Cry1Ac protein has been created to manage the giant borer, *Telchin licus*, showing effective pest control throughout different stages of plant development (Sakuno et al. 2024). The *Pinellia pedatisecta* agglutinin (PPA) gene, expressed in sugarcane, confers resistance to the sugarcane woolly aphid by modifying stomatal patterns and boosting antioxidant activity (Zhao et al. 2022). The expression of the Cry1Ab-Cry1Ac gene fusion via *Agrobacterium*-mediated transformation has led to the development of a resistant variety against the shoot borer (*Scripophaga excerptalis*). Results demonstrated that transgenic lines exhibited a decreased healthiness of the borer compared to the wild type (Koerniati et al. 2020). The particle bombardment of the cry2A gene cassette into the sugarcane cultivar ROC22 led to transgenic lines showing reduced susceptibility to stem borer infestations while achieving similar sugar yields (Gao et al. 2018).

17.4.2.6 Biomass and Bioethanol Production

Transgenic sugarcane represents a significant advancement in boosting bioethanol production, achieved through various genetic modifications to enhance biomass quality and yield (Verma et al. 2022c, d, 2024e, f). Recent advancements in genome editing, mainly through the application of CRISPR/Cas9 technology, have facilitated the reduction of lignin content in sugarcane while preserving or enhancing biomass yield, which is essential for sustainable biofuel production (Khan et al. 2019; Verma et al. 2022e, f, 2023b; Laksana et al. 2024). The application of RNA interference technology for gene silencing in sugarcane, focusing on the caffeic acid O-methyltransferase (COMT) gene, aims to improve biofuel production, especially bioethanol (Murugan et al. 2021). The *Agrobacterium*-mediated transformation that led to the overexpression of the SoSPS1 gene resulted in enhanced SPS activity and sucrose content in the leaves of transgenic sugarcane. Additionally, certain transgenic lines exhibited a notable increase in plant height and stalk number compared to the non-transgenic control (Anur et al. 2020). The use of RNA interference to reduce lignification in sugarcane, even within the context of its complex and highly polyploid interspecific hybrid genome, effectively decreases the recalcitrance of sugarcane biomass while maintaining plant performance under controlled environmental conditions (Jung et al. 2012; Verma et al. 2022g, 2023c, d). Genetic modification has the potential to lower lignin content and improve saccharification efficiency. By employing CRISPR–Cas9 gene editing to knock out the expression of an endogenous gene involved in lignin monomer biosynthesis, biomass yield can be significantly enhanced (Yu et al. 2021).

17.5 Regulatory and Biosafety Considerations

Assessing the environmental and biosafety aspects of transgenic sugarcane is essential for ensuring safe commercialization and addressing public concerns. The commercialization of transgenic sugarcane encounters numerous regulatory hurdles in various nations, particularly related to environmental safety, health evaluations, and market conditions. These challenges involve thorough assessments and approvals for any commercial launch. In India, the regulation of genetically modified organisms (GMOs) and their products falls under the "Rules for the manufacture, use, import, export & storage of hazardous microorganisms, genetically engineered organisms or cells, 1989" (commonly known as Rules, 1989). This framework is established under the Environment (Protection) Act of 1986, which serves as a comprehensive legislative foundation to safeguard and enhance the environment (Ahuja 2018). In South Africa, the GMO Act (Act 15, 1997) stipulates the necessity of a Trial Release Permit for field trials, which entails comprehensive monitoring and reporting protocols. The current status of commercial release applications is suspended, pending the resolution of outstanding issues related to intellectual property and market implications (Meyer and Snyman 2011). In Australia, the approval of

GM sugarcane is regulated by The Gene Technology Act 2000, which requires the completion of Risk Assessment and Risk Management Plans (RARMPs) before any environmental release. There have been no submissions for commercial release applications (Mitchell 2011). In Brazil, the regulatory process entails a comprehensive analysis of GM and non-GM sugarcane, emphasizing environmental and health implications. This comprehensive analysis is crucial for making well-informed choices about commercial launches.

17.6 Future Prospects and Challenges

The genomics of sugarcane is set to significantly improve crop productivity and sustainability by utilizing advanced methods such as pangenomics and gene editing. The integration of these technologies holds the potential to tackle the complex genetic issues linked to sugarcane, paving the way for enhanced traits and greater resilience to environmental pressures. Pangenomics offers a comprehensive perspective on the genetic diversity present in sugarcane, aiding in the discovery of essential genes for focused enhancements (Ali et al. 2023). Despite notable advancements in the development of molecular markers and bioinformatics tools for sugarcane, challenges persist due to its intricate polyploid genome, which impedes the swift application of genomic tools compared to other crops (Healey et al. 2024). RNA-seq provides notable benefits for examining gene expression but also presents challenges, including the necessity for reliable bioinformatics tools to interpret intricate datasets and the risk of biases in sequencing techniques. The findings highlight the significance of metabolic pathways and genetic factors in improving sugarcane resilience. However, it is crucial to consider the possible trade-offs between disease resistance and sugar yield, as specific resistance mechanisms might redirect resources from sugar production. Enhanced sugarcane varieties have the potential to significantly increase biomass yield, thereby promoting sustainable biofuel production and improving economic returns (Ahmad and Ming 2024). Creating climate-resilient cultivars using cutting-edge genetic techniques can alleviate the effects of climate change on sugarcane production (Surya Krishna et al. 2023). Concerns abound about transgenic sugarcane, fueled by apprehensions related to health risks and environmental consequences. Transgenic sugarcane presents notable benefits regarding yield and resilience; however, it is essential to tackle the challenges related to public perception and regulatory obstacles to promote its worldwide acceptance. The integration of genomics and transgenic breeding techniques in the advancement of sugarcane breeding has the potential to significantly improve crop resilience and productivity.

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Chapter 18
Developing Disease Resistance in Sugarcane: A Pathologists' Perspective

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Abstract About two-thirds of global sugar production depends on sugarcane, a major commercial crop in many tropical countries. Sugarcane production is threatened by various diseases caused by fungi, bacteria, phytoplasma and viruses. The severity and occurrence of the diseases depend on various factors, viz., host genotype, the prevalence of the pathotype, soil, temperature, humidity, etc. It is one of the most complex polyploidy crops, wherein the complexity is exacerbated with around 10–13 sets of homologous chromosomes. Hence, deciphering the complete genome of sugarcane remained a herculean task. While pathologists worldwide are constantly working to understand the molecular basis of disease resistance with various sugarcane genotypes and pathogens, breeders continue to improve the genetic makeup and develop commercially superior sugarcane varieties specific to various agro-climatic conditions through conventional breeding approaches. In this context, this chapter highlights the understanding of sugarcane disease resistance with a major focus on two critical diseases, viz., red rot and smut, at the molecular level and presents how the disease resistance in sugarcane can be enhanced from the perspective of sugarcane pathologists.

Keywords Disease resistance · Sugarcane · Pathologist · Red rot · Smut · Crop protection

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18.1 Introduction

Sugarcane contributes to around 70% of world sugar production, and it also serves as the major source of biomass for producing biofuel (ethanol) and many other value-added products. Among the various factors affecting the sugarcane productivity, biotic stress accounts for over 20 % yield loss. Over 200 diseases have been reported in sugarcane, and the impact of each disease varies geographically according to the varieties under cultivation, prevalent pathotypes and existing climatic conditions. Globally, red rot, smut, wilt, ratoon stunting disease (RSD), and brown rust are the few major diseases that hamper worldwide sugarcane production. In India, red rot and smut, the two major fungal diseases caused by *Colletotrichum falcatum* and *Sporisorium scitamineum*, respectively, pose a major threat to productivity. So, in the Indian breeding program, screening for disease resistance against these two diseases is one of the essential criteria for commercial release. However, with the conventional breeding and screening approaches, it takes around 14 years from breeding to commercial varietal release of sugarcane varieties.

18.2 Intricacies of Molecular Breeding for Disease Resistance in Sugarcane

For centuries, sugarcane has been domesticated from wild grass species to potential food, fodder and energy crop with repeated crossing, back-crossing, and continuous hybridization to obtain commercial varieties with superior agronomic traits like high sucrose, yield, disease resistance, etc. (Souza et al. 2011; Sundar et al. 2015; Dinesh Babu et al. 2022). This process of anthropogenic evolution or species introgression of sugarcane has led to phenomenal genomic rearrangements in modern hybrid cultivars and increased the complexity of this polyploidy crop, which makes it challenging to decipher its whole genome of approximately 10 Gb with 80–130 chromosomes, even with the advanced high-throughput genomic sequencing technologies (Piperidis and D'Hont 2020; Ashwin et al. 2022; Healey et al. 2024). Understanding the molecular basis of sugarcane disease resistance is challenging due to the absence of genomic information till 2024. Therefore, the molecular breeding approaches for disease resistance are still in the infancy, as far as sugarcane is concerned. Whatever modest milestones achieved with molecular breeding for specific traits in specific genotype(s) may not work well with other populations, because of the extreme polymorphism among the 8–15 uneven homo(eo)logous loci in the autopolyploidy genome (Chen et al. 2023; Lu et al. 2024). Consequently, genome mapping or identification of disease-resistance loci has become a challenge in sugarcane, which is otherwise relatively more straightforward in other crops.

Meanwhile, the advent of high-throughput technologies of omics have revolutionized the understanding of host plant resistance and pathogen biology. Notably, the past two decades have witnessed a tremendous transformation of understanding

of the molecular aspect of plant–pathogen interactions, including sugarcane–pathogen interactions (Ashwin et al. 2017a, 2020a; Sundar et al. 2018; Healey et al. 2024). This holistic understanding of plant–pathogen interaction has a leveraging effect on understanding core plant disease resistance, especially in the case of sugarcane, where genomic information is lacking (Ashwin et al. 2022). Hence, the term disease resistance, thus viewed by breeders and plant biotechnologists, differs from a plant pathologist's perspective. For instance, besides the various environmental cues like the soil, soil moisture, temperature, humidity, etc., the degree of disease resistance being conferred against a specific pathotype by a sugarcane genotype is extrinsically and intrinsically influenced by various factors. Extrinsic factors include the type and range of effectors and toxins of the pathogen that aid in evading and colonizing the pre-formed and induced defenses of the host, respectively. Intrinsic factors include the resistance conferred by structural barriers, expression of resistance genes/proteins, and metabolites (Hidayah et al. 2021).

18.3 Leveraging the Evolving Continuum of Understanding of Plant–Pathogen Interactions

In general, our understanding of disease resistance has undergone a paradigm shift in the past decade due to the emerging concepts in molecular plant–pathogen interactions. Historically, the concept of plant–pathogen interaction at the molecular level has evolved with respect to the molecular players involved and the level of resistance it confers. Various concepts or models were proposed from the previous century to the present decade to explain the evolving understanding at a protein–protein level from the gene-for-gene concept of H. H. Flor in the 1940s to the guard hypothesis (van der Biezen and Jones, 1998), the concept of decoy model (van der Hoorn and Kamoun, 2008) and the most recent iceberg model (Thordal-Christensen 2020). Decoding the paradigm of molecular events of this interaction at the protein–protein level has reiterated the significance of the molecular players like PAMPs, effectors, receptors, R proteins and their interacting partners, and subsequent signal transduction processes that result in the selection pressure and co-existence among plant–pathogen interactions in the ecosystem (Ashwin et al. 2020a; Noman et al. 2021).

Plants perceive the presence of pathogens through their conserved molecular signatures called pathogen-associated molecular patterns (PAMPs) and induce PAMP-triggered immunity (PTI) (Defalco and Zipfel 2021). When the pathogens try to evade PTI by secreting a repertoire of effectors, plants employ a range of receptor proteins called pattern recognition receptors (PRRs) at the cell surface. In the meantime, the pathogens secrete a secondary wave of potent effectors to suppress the PTI and colonize the plant host. This second layer of attack is encountered by intracellular receptors or R proteins, which recognize and activate effector-triggered immunity (ETI) (Wu et al. 2014). Extrinsically, these PAMPs can also

potentially act as resistance inducers and trigger the host's active resistance by mimicking the pathogen's perception without a real-time confrontation (Burketova et al. 2015). Nevertheless, specific effectors can also induce host resistance when the host genotype is endowed with concomitant receptors, which can then be designated as elicitors or inducers of disease resistance (Abdul Malik et al. 2020). The active resistance thus induced with these elicitors would be systemic, durable, and broad spectrum, the efficacy of which can range from 20% to 85% (Walters et al. 2013; Flors et al. 2024).

Overall, the innate and induced resistance phenomena indicate that all the plants have a basal defense system with a range of receptors and their direct and indirect interacting partners from the surface to the cytoplasmic level to counteract pathogen invasion. Besides these receptors and their associated interacting proteins, plants deploy a range of antimicrobial proteins and metabolites, which can also form part of pre-formed (passive) defense or active defense to counter-attack the colonization. Hence, the source of resistance becomes multigenic in nature in most of the cultivated crops, especially in the case of sugarcane, wherein the genome is polyploidy, and the gene expression level is regulated by multiple factors at various levels—epistatical, transcriptional, post-transcriptional and at translational levels. Moreover, such complex regulation is also not uniform among hybrid cultivars. Though all these complexities add to the challenges, the recent decoding of the sugarcane genomic architecture by Healey et al. (2024) has opened up new vistas. It raises hope that the future looks more promising as far as deciphering disease resistance in sugarcane is concerned.

18.4 Molecular Insights into Disease Resistance in Sugarcane

The present understanding of the molecular basis of sugarcane disease resistance is not entirely encouraging due to various hurdles associated with its genomic complexity, as stated above. Gradual progress is evident with the paucity of available literature on elucidating defense pathways or identifying disease resistance markers in sugarcane. Even though preliminary molecular analyses on sugarcane disease resistance started a few decades back, it has acquired momentum only in the past one and half decades. Hitherto, only a single R gene has been identified as a disease-resistance gene in sugarcane called Bru1. It has been identified as an R gene for brown rust caused by *Puccinia melanocephala* and has been experimentally proven (Costet et al. 2012; Correr et al. 2020). Krattinger et al. (2011) have identified the homologous EST clusters that encode Lr34 in sugarcane. Lr34 is a wheat gene encoding for ABC transporter that reduces the early stage of haustorial formation in *Puccinia triticana*, causing leaf rust. Que et al. (2009) identified the presence of non-TIR-NBS-LRR type resistance genes in the genome of sugarcane during interaction without fungi—*S. scitamineum*. Despite information on smut and brown rust resistance, there is no clear understanding of the PTI/ETI signaling mechanisms in these pathosystems. Besides, SUGARWIN2, a defense in a class of sugarcane

proteins, has been reported to induce cell death in *C. falcatum* under in vitro conditions (Franco et al. 2014).

18.4.1 Red Rot

In India, various research groups have attempted to understand the red rot and smut resistance at the molecular level. Comparative analysis of incompatible and compatible interactions of Sugarcane- *C. falcatum* showed early upregulation of chitinases and β-1,3-glucanases, metallothionein, a resistance gene analogue (RGA) R30 and a receptor kinase (Viswanathan et al. 2005; Rahul et al. 2015). Prathima et al. (2013) identified 112 ESTs, which were potentially regulated during compatible interactions using DD-RT-PCR, followed by (Sathyabhama et al. 2015), wherein suppression subtractive hybridization (SSH) and qRT-PCR revealed early and higher expression of many defense-related genes viz., β-1,3-glucanase, PR10 genes, Ser/Thr protein kinase receptor precursor, NBS-LRR genes, etc. in incompatible interaction (Sathyabhama et al. 2022). An association mapping study by Singh et al. (2016) identified many defence genes, viz. cytochrome P450, MAP Kinase-4, Ser/Thr protein kinase, Glycerol-3-phosphate transporter-1, etc., that are possibly associated with red rot resistance. Recently, eight miRNAs and their targets were identified in sugarcane, likely to confer resistance to red rot for the first time, highlighting the miRNA-mediated defense mechanism in sugarcane (Nandakumar et al. 2021a). Also, the role of 3-deoxyanthocyanidin phytoalexins in red rot resistance has been identified, and the study elucidated a few phytoalexin pathways, that are highly upregulated in red rot-resistant varieties (Nandakumar et al. 2021b).

Meanwhile, a handful of reports are available on the front of induced resistance for the past two decades. The molecular basis of induced resistance (IR) has been demonstrated with the induction of an array of defense-related compounds in response to BTH and salicylic acid (SA) priming (Ramesh Sundar et al. 2001, 2006, 2012). Similarly, a glycoproteinenaceous elicitor called Cf elicitor purified from the cell wall of *C. falcatum* induced the production of many defense-related PR proteins and suppressed the degree of pathogen colonization (Ramesh Sundar and Vidhyasekeran 2003; Ramesh Sundar et al. 2002a, b, 2008, 2009). Further, transcriptional profiling of systemic acquired resistance response showed that BTH, SA, and Cf elicitor treatments upregulated the expression of several defense-related transcription factors (TFs) (Muthiah et al. 2013; Ashwin et al. 2017b), phenylpropanoid pathway genes and resistant gene analogues (RGAs), including NBS-LRR proteins at earlier stages of rot infection (Selvaraj et al. 2014). In continuation of the exploration of biotic resistance inducers via the comparative secretomics approach, Ashwin et al. (2017c) identified a pathogen-associated molecular pattern (PAMP) of *C. falcatum* (CfEPL1) and a few other potential effectors. For the first time, it has demonstrated the potential role of CfEPL1—eliciting plant response-like protein in inducing PAMP-triggered immunity (PTI) in sugarcane. This CfEPL1, a ceratoplatanin protein class, possesses versatile biophysical properties and plays significant

biological roles in plant–microbial interactions (Ashwin et al. 2023). Similarly, the same group has identified a novel low molecular weight cysteine-rich secreted protein of *C. falcatum* called CfPDIP1. This protein was reported to possess defense elicitation properties in sugarcane and trigger hypersensitive response (HR) in tobacco. Notably, it has been predicted as a potential effector of *C. falcatum* by *in silico* analysis (Ashwin et al. 2018). Further, the transcriptome analysis targeting candidate secretory effector proteins (CSEPs) from *C. falcatum* (Naveen et al. 2019) has identified a few small secreted proteins as key pathogenic determinants. Deciphering the effectorome of *C. falcatum* and integrating the IR approach would aid in developing a potential pathogen-derived disease resistance strategy against a broad spectrum of pathogens in sugarcane. Further, the information generated on the interactome of Sugarcane-*C. falcatum* interaction would also provide a deeper insight into the sequential events that orchestrate the defense mechanism, including PTI and ETI responses.

On the host side, the expression profiling of transcripts coding for major defense-signaling pathway regulatory genes during compatible and incompatible interactions indicated major defense-signaling pathways, especially SA pathway-associated genes (Ashwin et al. 2020b). Notably, the non-expression of pathogenesis-related gene 1 (NPR1), the master regulator of defense pathways, was highly upregulated in incompatible interactions than the compatible interaction. Overall, the study highlighted that SA pathway genes are highly reprogrammed in response to BTH, CfEPL1 and CfPDIP1 priming and provided deeper insights into how the induced resistance is being operated in resistant and susceptible sugarcane varieties.

18.4.2 Smut

Since 2000, researchers have relied on marker-based approaches for screening of smut resistance. Amplified fragment length polymorphism (AFLP), cDNA-AFLP, simple sequence repeats (SSRs), and single-nucleotide polymorphism (SNP) array were used to identify the markers associated with smut resistance/susceptibility (Sundar et al. 2012). For instance, initial attempts to identify quantitative trait loci of smut resistance with AFLP did not result in promising markers (Raboin et al. 2003). Later on, Wei et al. (2006) employed AFLP and SSR markers against 154 sugarcane clones and identified 11 markers associated with smut susceptibility and resistance. Similarly, Gouy et al. (2015) screened 183 sugarcane genotypes with 3327 AFLP markers and identified few markers associated with smut resistance with linear models. However, Aitken et al. (2016) used an Axiom SNP array with 24,297 polymorphic SNPs against 367 sugarcane cultivars/genotypes and successfully narrowed it down to 1259 markers associated with smut resistance and 54 markers with the highest probability.

Meanwhile, next-generation sequencing platforms have been employed worldwide to understand transcriptional changes during real-time plant–pathogen interactions in the past decade. Sugarcane smut, being a disease of global prevalence, has

attracted several research groups across the world, and the whole genome of *S. scitamineum* has been deciphered by many groups. By employing RNAseq, Schaker et al. (2016) reported a significant alteration in the transcriptional abundance following whip development in sugarcane, and the study served as a hypothetical model to understand the progression of sugarcane smut colonization. Similarly, McNeil et al. (2018) studied smut resistance mechanisms using RNA-seq and the results indicated that the early sugarcane response to *S. scitamineum* infection is complex. The study reported that many external barriers associated with molecular processes are involved in the early stage of colonization, and the endogenous cellular defense mechanism gets activated upon further progression in colonization. Further, few RNA-seq studies attempted to decipher the molecular events of sugarcane–smut interaction and found many sugarcane cultivars exhibit non-specific, but strong defense response against smut colonization (Marques et al. 2018; McNeil et al. 2018; Su et al. 2016; Bedre et al. 2019). Similarly, proteomics-based studies on sugarcane–*S. scitamineum* interactions and pathoproteomics indicated that phenylalanine metabolism, flavonoid/lignin/phenylpropanoid biosynthesis and ribosomal and peroxisomal associated pathways are associated with smut resistance (Barnabas et al. 2015, 2016, 2017; Su et al. 2016; Peters et al. 2020; Kumaravel et al. 2024).

Recently, a comparative whole transcriptome profiling has been completed for high and low-virulent Indian isolates during interaction with the host (Agisha et al. 2022a), and the data analysis has generated interesting insights into the mechanism of pathogenicity and host resistance. Meanwhile, a global transcriptional reprogramming even was deciphered during the lifestyle transition of *S. scitamineum* - from non-infective hyphae to infective fused dikaryotic myceleium (Agisha et al. 2022b). Unravelling the molecular events during this phase under *in vitro* and *in planta* conditions aids in the identification of the regulatory master switches, which mediates the virulence/pathogenicity and variability of *S. scitamineum* (Barnabas et al. 2018; Nalayeni et al. 2021; Vinodhini et al. 2023). Recently, Chen et al. (2023) also found that antagonistic microbes and endophytes may contribute to differential response to smut resistance.

18.5 Conclusion

The massive paradigm shift in understanding plant disease resistance in plants is now evident with the rapidly evolving robust molecular biology platforms. Integrated tools of "Omics," namely genomics, proteomics, metabolomics, etc., have transformed the fundamental concepts of plant disease resistance in many crops. Especially the high throughput platforms of genomic tools and the recently evolved *de novo* biomolecular identification algorithms in proteomics and metabolomic platforms have taken the research on disease resistance to the next level. Though the progress in sugarcane is slow due to the obstacles in untangling its inherent genomic complexity, pathologists and biotechnologists are making considerable

progress. It can be extended further by employing the integrated "omic" tools. With the emergence of new technologies day by day, a landmark achievement has been made with the very recent genome sequencing of sugarcane. Also, the same integrated tools of "omics" would portray the complete picture of the entire cascade of molecular events behind the disease resistance mechanism in different sugarcane cultivars. With recent landmark progress in unwinding the sugarcane genome and many pathogens in the pipeline, the future looks even more promising in comprehensively deciphering disease resistance in sugarcane.

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Chapter 19
Innovative Breeding Insights to Enhance Tolerance Towards Sucking Pests of Sugarcane

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Abstract Sugarcane (*Saccharum* spp. Hybrids) plays an essential role globally as a source of sugar and biofuel. However, its yield potential is frequently threatened by biotic stress, especially sucking insect pests such as aphids, hoppers, mealybugs, scale insects, and whiteflies, which have been alarming in recent years. These pests diminish yield and sucrose quality and serve as vectors for plant diseases, amplifying substantial economic losses. Traditional breeding approaches have improved sugarcane yield and pest resistance over the years. Nevertheless, challenges persist because of its intricate polyploid genome and restricted genetic diversity, which hinder the development of resistant varieties. Recent innovations in sugarcane breeding now incorporate molecular and biotechnological tools, notably CRISPR, RNA interference (RNAi), and marker-assisted selection (MAS) to enhance pest resistance traits. CRISPR and RNAi enable precise gene manipulation, targeting pest-vulnerable genes, while MAS allows efficient identification and integration of pest-resistant traits into new varieties. These methods collectively offer a sustainable alternative to conventional pesticide reliance, minimizing environmental

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impact and improving crop resilience. Nonetheless, integrating these advanced tools into practical breeding faces technical, regulatory, and cost-related challenges, which must be addressed for effective implementation in resistance varietal development programs. The chapter provides insights into how these novel breeding techniques can be harmonized with traditional crop breeding to develop sugarcane-resistant varieties to mitigate the impact of sucking pests, ultimately contributing toward sustainable and resilient sugarcane cultivation.

Keywords Sugarcane breeding · Sucking insects · Pest resistance · Molecular tools

19.1 Introduction

Sugarcane (*Saccharum* spp.), belonging to the Poaceae family, is a vital cash crop cultivated in numerous tropical and subtropical regions. It serves as a primary source of sugar, by-products, and bioenergy worldwide (Verma et al. 2024a, b). Sugarcane is grown on approximately 26 million hectares across more than 90 countries, with a global production of 1.83 billion tons (FAOSTAT 2023). Following Brazil, India ranks as the world's second-largest sugarcane producer (Satyagopal et al. 2014; Chen et al. 2020). The demand for sugar and sugarcane-derived by-products continues to rise in domestic and industrial markets. To meet this growing demand, sustainable improvements in sugarcane yield are essential (Verma et al. 2019a, b; Bhatt et al. 2024). High yield is the ultimate objective of plant breeding, including sugarcane. Sugarcane cultivation is heavily impacted by various biotic stressors, which are the primary drivers of fluctuations in sugar production (Rao 1970; Kumarasinghe and Wratten 1996; Joshi and Viraktamath 2004; Srikanth 2004; Kumar et al. 2006; El-Dein et al. 2009; Filho et al. 2019; Geetha et al. 2022; Verma et al. 2022a, b; Ambethgar et al. 2024; Kumar 2024; Li et al. 2024). Diseases, pests, nematodes, and weeds are vital biotic stressors that significantly impact the growth, development, and overall performance of sugarcane plants (Filho et al. 2019; Mahesh et al. 2019; Song et al. 2019; Singh et al. 2019; Omkar and Tripathi 2020; Geetha et al. 2022; Ambethgar et al. 2024; Monteiro et al. 2024).

Every part of sugarcane endures attack from one or more pests from planting to harvesting (Srikanth et al. 2011). In recent years, sugarcane crops have faced substantial challenges due to infestations by a complex of sucking pests, including woolly aphids, sugarcane aphids, hoppers, mealybugs, whiteflies, scales, and mite species, all pose a significant threat to sugarcane crops by draining cellular sap, eventually reducing yield, sucrose content, and overall plant health (Kumarasinghe and Wratten 1996; Allsopp and Manners 1997; Joshi and Viraktamath 2004; Srikanth et al. 2012; Filho et al. 2019; Singh et al. 2020a; Verma et al. 2024c; Afghan et al. 2024). In tropical and subtropical regions, where sugarcane is primarily

cultivated (Bhatt et al. 2023a, b, 2024), infestations by sucking pests are increasingly worsened by the effects of climate change, often leading to colossal economic losses (Ambethgar 1996; Srikanth et al. 2012; Dhaliwal et al. 2015; Filho et al. 2019; Mahesh et al. 2019; Omkar and Tripathi 2020; Geetha et al. 2022; Jagadesh et al. 2024; Ambethgar et al. 2024; Monteiro et al. 2024). The overreliance on chemical pesticides to manage these pests is widespread, yet frequent applications raise concerns over environmental impact, human health, pest resistance, and escalated production costs (Ambethgar 1996; Joshi and Viraktamath 2004; Dhaliwal et al. 2015; Filho et al. 2019; Kumar et al. 2021; Huang et al. 2024). The harmful impact of major sucking insect pests on sugarcane is discussed.

In this context, developing pest-resistant sugarcane varieties is essential for a more sustainable approach to the pest management system (Joshi and Viraktamath 2004; Geetha et al. 2022; Ambethgar et al. 2024). Incorporating natural resistance into sugarcane cultivars offers a stable solution, reducing the need for chemical inputs, and providing long-term benefits to cane farmers and the environment (Geetha et al. 2022; Verma et al. 2022a, b; Chen et al. 2024; Liang et al. 2024). Traditional breeding programs have focused on identifying and crossbreeding naturally resistant varieties, but the complex polyploid nature of sugarcane presents challenges in achieving durable resistance (Jackson et al. 2022; Guo et al. 2021, 2022; Oliveira et al. 2022). Additionally, there are inherent challenges in breeding sugarcane due to its long growth cycle and the labor-intensive screening process for pest-resistance traits (Belide et al. 2017). Genetic and biotechnological advancements are currently being utilized to select superior, multipurpose agro-industry cultivars (Verma et al. 2022a, b; Huang et al. 2022; Chen et al. 2023).

Recent advancements in molecular breeding, such as marker-assisted selection (MAS) and genetic mapping, have made it possible to more precisely introduce resistance traits into sugarcane (Tena et al. 2014; Aslam et al. 2018; Geetha et al. 2022). These tools assist in identifying and integrating specific genes associated with resistance, thereby enhancing the efficiency of breeding programs, particularly in developing pest-resistant varieties (Singh et al. 2020a, b; Geetha et al. 2022). Transgenic approaches and novel techniques like CRISPR-Cas9 gene editing are also being explored to target specific genes associated with pest resistance, potentially overcoming the limitations of conventional breeding methods (Weng et al. 2023). This review summarizes innovations in sugarcane breeding for pest resistance, focusing on both classical and molecular techniques to counteract threats inflicted by sucking pests.

19.2 Biotic Stress: Impact Due to Sucking Pests

Sugarcane production is continuously impacted by various biotic stresses (Azevedo et al. 2011; Song et al. 2021, 2022). The acceleration of climate change, the expansion of sugarcane monoculture farming, and the heavy reliance on chemical products have increased the likelihood of frequent pest outbreaks (Kumar, 2024; Kumar

and Gaikwad 2017; Geetha et al. 2022; Ambethgar et al. 2024). Sucking insects are a severe and destructive group of pests affecting sugarcane crops in India (Bhavani and Narasimha Rao 2013; Bhargava et al. 2020; Geetha et al. 2022; Ambethgar et al. 2024). Sucking pests belong to a large clan of insects called Hemiptera, within the suborders Heteroptera (true bugs) and Homoptera (aphids, leafhoppers, mealybugs, scales, and whiteflies), which are characterized by incomplete metamorphosis, with three distinct life stages: egg, nymph, and adult (Dhaliwal et al. 2015; Chand et al. 2017; Cheralu et al. 2018). Sucking pests, for example, the mealybugs, pierce plants with their slender, sharp mouthparts to suck out the essential plant sap (Fig. 19.1).

The sucking insects harm the crops by extracting the sap from them, thereby weakening the plants and transmitting several bacterial, fungal and viral pathogens (Ambethgar 1996; Joshi and Viraktamath 2004; Mahesh et al. 2019; Geetha et al. 2022). The extraction of cellular sap leads to discolored spots or scars on the leaves or stems of the sugarcane plant (Mahesh et al. 2019; Malviya et al. 2020; Geetha et al. 2022). It may also cause senescence, deformed leaves, and dry entire plants under pest severity. A list of sucking pests with their systematics is provided in Table 19.1.

Many of these pests have developed resistance to pesticides (Joshi and Viraktamath 2004; El-Dein et al. 2009; Dhaliwal et al. 2015; Mahesh et al. 2019; Geetha et al. 2022; Ambethgar et al. 2024). Some of these sucking insects are vectors, transmitting a range of plant viruses or phytoplasma diseases that affect sugarcane crops. The leafhoppers *Matsumuratettix hiroglyphicus* and *Yamatotettix flavovittatus* serve as vectors for the phytoplasma responsible for sugarcane white leaf (SCWL) disease. As for sugarcane mosaic disease (SCMV), aphids such as *Dactynotus ambrosiae, Hysteroneura setariae, Longiunguis sacchari, Rhopalosiphum maidis*, and *Toxoptera graminum* act as its vectors. Ants can

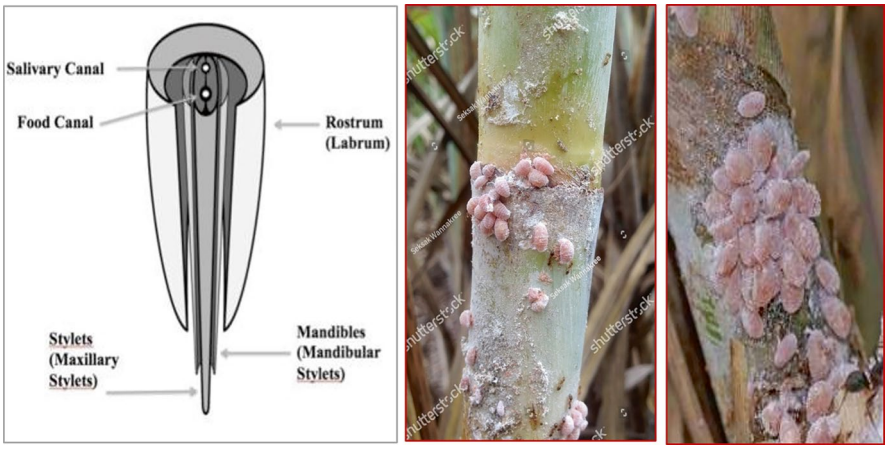

Fig. 19.1 Feeding mechanism of sucking insects with sharp-pointed mouthparts [Source: www.wikipedia]

Table 19.1 A list of sucking insect pests that damage sugarcane, *Saccharum officinarum* L.

Common name	Scientific name	Family	Order	Pest status
Pink mealybug	Saccharicoccus saccharin Cockerell	Pseudococcidae	Hemiptera	Major
Crown mealybug	Pseudococcus saccharifolii Green	Pseudococcidae	Hemiptera	Major
Mealybug	Ripersia saccharin Green	Pseudococcidae	Hemiptera	Major
Whitefly	Aleurolobus barodensis Maskell	Aleyrodidae	Hemiptera	Major
Woolly aphid	Ceratovacuna lanigera Zehntner	Aphididae	Hemiptera	Major
Yellow aphid	Sipha flava Forbes	Aphididae	Hemiptera	Major
Leafhopper	Pyrilla perpusilla Walker	Lophopidae	Hemiptera	Major
Planthopper	Perkinsiella saccharicida Kirkaldy	Delphacidae	Hemiptera	Major
Scale insect	Acanthomytilus saccharin Hall	Diaspididae	Hemiptera	Minor
Scale insect	Aclerda takahashii (Kuwana)	Diaspididae	Hemiptera	Minor
Scale insect	Duplochinaspsis divergence Green	Diaspididae	Hemiptera	Minor
Scale insect	Melanaspis glomerata Green	Diaspididae	Hemiptera	Major
Scale insect	Odonaspis saccharicaulis Zhnt.	Diaspididae	Hemiptera	Minor
Scale insect	Temnaspidiatus kellyi Brein.	Diaspididae	Hemiptera	Minor
Black bug	Blissus gibbus Fab.	Lygaeidae	Hemiptera	Minor
Black bug	Macropes excavates Distant	Blissidae	Hemiptera	Minor

indirectly spread SCMV by interacting with aphids in infected sugarcane fields. Virus-infected plants cannot be cured.

19.3 Mechanisms of Host–Plant Resistance

Pest and disease resistance refers to an organism's inherited ability to counteract the effects of an insect, pathogen, or other harmful factors, either partially or completely. Resistance is demonstrated when symptoms are minimal or absent, indicating that the pest is unable to spread or spreads with difficulty on the host (Khush and Brar 1991; Ahman 2006; Li-Hong et al. 2006; Price and Gatehouse 2008; Azevedo et al. 2011). The resistant traits of plants are typically due to their morphological and biochemical characteristics, which influence the insect's behavior and biology, ultimately promoting better survival and productivity of the crop plants (Khush and Brar 1991; Wu et al. 2022). The plant's resistance level can be determined by comparing resistant plants to more susceptible ones under identical conditions (Singh et al. 2021). Naturally occurring pest-resistant traits in plants can be classified as induced or constitutive resistance (Li-Hong et al. 2006; Price and Gatehouse 2008). Induced resistance occurs when the plant detects an invader, prompting it to produce proteins or metabolites that are detrimental to the intruder (Khush and Brar 1991; War et al. 2012).

In induced resistance, the plant identifies the pest through at least one molecule produced by the pest. This molecule can be a protein, a fatty acid derivative (such as fatty acid-amino acid conjugates), or another chemical compound secreted by the

pest (Khush and Brar 1991). Constitutive resistance refers to the inherent level of resistance in the plant, which is not triggered by pest attack. It can manifest in morphological, structural, or chemical forms (Khush and Brar 1991; Azevedo et al. 2011). Arimura et al. (2005) categorized resistance into direct and indirect defenses. Direct defenses immediately affect herbivores attacking the plant, including physical barriers like thorns, trichomes, waxes, and spines, as well as chemical defenses involving secondary plant metabolites or specialized defense proteins. These direct defenses can be constitutive or inducible (Azevedo et al. 2011). Indirect defenses include interactions with other species, such as natural enemies of the insect pest. Plant resistance consists of three components: antixenosis, antibiosis, and tolerance. A resistant plant may exhibit one or more of these mechanisms, but it is most beneficial for all three to be present in a resistant variety (Khush and Brar 1991; Ahman 2006; Azevedo et al. 2011). A more detailed discussion of these mechanisms will be provided in the following sections of this article.

19.4 Components of Host–Plant Resistance

In his seminal book *Insect Resistance in Crop Plants*, Painter (1951) proposed three components of resistance: antixenosis, antibiosis, and tolerance. These components can be either physical or chemical in nature, or a combination of both and are briefly outlined below:

19.4.1 Antixenosis

Antixenosis is a resistance mechanism in plants that can be morphological, physical, or structural, and it disrupts the behavioral activities of insects, such as mating, egg-laying, and feeding. As a result, the insect tends to avoid the host plant (Azevedo et al. 2011). This resistance may result from factors such as the color, texture of the leaf surface, specific alleles or chemicals, or a combination of these elements in the plant, which discourage the insect pest and prevent oviposition. Antixenosis has sometimes been utilized to develop resistant crop varieties (Kumarasinghe and Jepson 2003; Azevedo et al. 2011; Srikanth et al. 2011). Antixenosis involves plant traits that deter insects from a particular host, which can be displayed in a cultivar through morphological characteristics or allelochemicals. This type of resistance influences insect behavior, making the plant less appealing for egg-laying, feeding, or shelter. The lack of physical or chemical cues essential for host selection or the presence of antifeedants, repellents and deterrents supports the antixenosis resistance mechanism (Kumarasinghe and Jepson 2003; Srikanth et al. 2012).

19.4.2 Antibiosis

Antibiosis refers to a plant's negative impact on an insect's growth and survival, influencing the insect's biology by increasing mortality or reducing its lifespan and reproductive capacity (Azevedo et al. 2011). Antibiosis negatively impacts the biology of an insect attempting to use the plant as a host and it can be either physical or chemical in nature. The damage to the insect can be significant, often affecting the larvae and eggs. Insects that survive the effects of antibiosis may experience stunted growth, delayed development and reduced fecundity. This resistance is usually linked to allelochemicals, growth inhibitors, and morphological traits that prevent insect attacks. For example, allelochemicals like glycoalkaloids in potato, γ-tomatine in tomato, gossypol in cotton and rutin, and chlorogenic acid in tomato are toxic to insects, leading to lower pest populations and reduced damage to resistant plants. Antibiosis is frequently associated with these allelochemicals (Kumar et al. 2006).

19.4.3 Tolerance

Tolerance is a plant's ability to endure or recover from insect damage, representing its response to such harm. Various factors contribute to tolerance, including enhanced plant vigor, compensatory growth, and wound healing (Reese et al. 1993). This mechanism enables the plant to continue normal growth and increase its biomass, regardless of the extent of insect infestations (Azevedo et al. 2011; Verma et al. 2020a, b; Joshi et al. 2022). Painter (1951) defined tolerance as "a form of resistance where the plant can grow, reproduce, or repair damage to a significant extent, even while supporting a pest population similar to that affecting a susceptible host." According to Reese et al. (1993), tolerance is the preferred resistance mechanism because it does not negatively affect natural enemy populations. In contrast, antixenosis and antibiosis increase selection pressure on insect populations, potentially leading to more virulent biotypes and detrimental effects on control methods.

19.5 Host–Plant Resistance Mechanism

Host–plant resistance refers to the characteristics of a plant that allow it to prevent, endure or recover from insect attacks, even under conditions that would cause more significant damage to other plants of the same species. According to Singh et al. (2020a, b), host–plant resistance is the combination of genetically inherited traits that enable a plant from one cultivar or species to experience less damage than a susceptible plant lacking these traits. Panda and Khush (1995) and Singh et al. (2020a, b) identified four key characteristics for evaluating plant resistance:

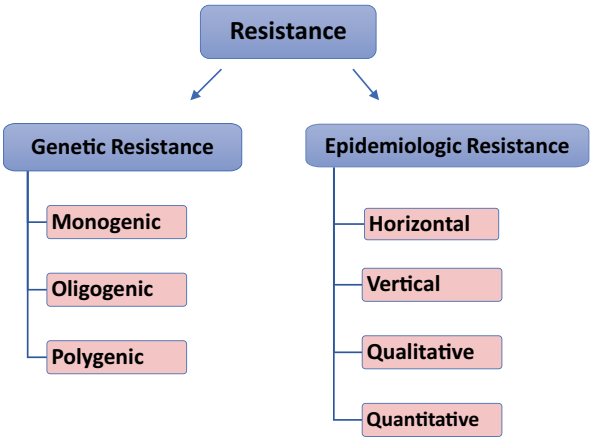

Fig. 19.2 Categories of crop resistance based on genetic and epidemiological characteristics

- Resistance is inheritable and governed by one or more genes
- It is assessed by comparing it with a susceptible cultivar of the same species
- Resistance can be quantified using standard scoring methods
- It is adaptable and may be influenced by environmental, biotic, and abiotic factors.

Resistance can be categorized based on genetic and epidemiological characteristics as illustrated in Fig. 19.2.

19.6 Genetic Resistance

Genetic resistance refers to the natural ability of a plant variety to withstand diseases or pests due to specific genes within its genetic makeup, allowing it to resist infection or damage from pests or pathogens, essentially acting as a defense mechanism without the need for external chemical treatments; this resistance can be either inherited from wild relatives or developed through selective breeding technique (Reese et al. 1993; Panda and Khush 1995; Azevedo et al. 2011; Singh et al. 2020a, b; Xu et al. 2022; Tian et al. 2023). Sugarcane has several genetic traits that make it resistant to diseases and pests. Genetic resistance can be grouped into the following types or categories.

19.6.1 Monogenic Resistance

Monogenic resistance in sugarcane refers to a situation where a single gene within the sugarcane plant provides resistance against a specific disease or pest, meaning the resistance trait is controlled by the inheritance of one gene, unlike polygenic

resistance, where multiple genes contribute to the trait; an example is the "Bru1" gene which confers resistance to brown rust in sugarcane, where many modern cultivars carry this single gene for resistance.

19.6.2 Oligogenic Resistance

Oligogenic resistance in sugarcane refers to a type of pest resistance where a relatively small number of genes, typically a few significant genes, control the plant's ability to defend against a pest or pathogen, providing a strong resistance but potentially vulnerable to rapid pest evolution if a single race of the pest or pathogen overcomes those genes; in contrast to polygenic resistance, where many genes contribute to a broader, more durable.

19.6.3 Polygenic Resistance

Polygenic resistance in sugarcane refers to a type of disease resistance where multiple genes across the plant's genome contribute to its ability to withstand a pathogen rather than relying on a single dominant resistance gene, meaning the resistance level is influenced by the combined effect of many small genetic variations, providing a more durable and broad-spectrum protection against diseases like sugarcane mosaic virus (SCMV) or its insect vectors.

19.6.4 Epidemiologic Resistance

Epidemiologic resistance in sugarcane refers to the ability of a sugarcane variety to withstand a widespread pest or disease outbreak within a particular region, meaning it can resist infection even when the pest is prevalent in the environment, effectively limiting the spread of the disease across a large population of sugarcane plants (Reese et al. 1993; Azevedo et al. 2011; Singh et al. 2020a, b). This resistance is typically achieved through genetic breeding programs that select for cultivars with inherent resistance traits against specific pests or pathogens.

19.6.5 Horizontal or Durable Resistance

In sugarcane, "horizontal resistance" is considered the more long-lasting form of resistance, as multiple genes control it and provide protection against a broad range of pathogen races, unlike "vertical resistance," which is governed by a few significant genes and can be easily overcome by evolving pathogen strains.

19.6.6 Vertical Resistance

Vertical resistance (VR) in sugarcane refers to the plant's ability to resist a specific race or strain of a pathogen. It is also referred to as physiological resistance, seedling resistance, perpendicular resistance, or race-specific resistance.

19.6.7 Qualitative Resistance

Qualitative resistance in sugarcane refers to a type of disease resistance controlled by a single gene, meaning a plant either has complete resistance to a pathogen or is completely susceptible, with a clear distinction between resistant and susceptible individuals, unlike quantitative resistance, where resistance levels vary along a spectrum.

19.6.8 Quantitative Resistance

Quantitative resistance in sugarcane refers to a type of disease resistance where a plant does not exhibit a clear "resistant" or "susceptible" response but instead shows a range of resistance levels depending on the genetic makeup, meaning the degree of infection varies across individuals, making it a continuous trait measured on a scale rather than a simple binary response; this type of resistance is often controlled by multiple genes and is considered more durable against pathogen evolution compared to qualitative resistance which is governed by a single major gene.

19.7 Resistance Breeding in Sugarcane

Modern sugarcane varieties are complex polyploid and aneuploid hybrids with exceptionally large genomes (over 10 GB), which have been developed through artificial crosses between the species *Saccharum officinarum* and *S. spontaneum*

(Panda and Khush 1995; Azevedo et al. 2011; Singh et al. 2020a, b). Any sugarcane breeding program must consider the plant's ability to adapt to biotic stressors. To comprehend the factors contributing to biotic stress resistance, it is essential to understand sugarcane taxonomy. Ongoing research on various wild species focuses on their potential to tolerate biotic stresses. The genetic complexity and low fertility of sugarcane in natural environments make conventional breeding methods highly labor-intensive, expensive, and time-consuming (Azevedo et al. 2011). Sugarcane's ability to propagate vegetatively, ensuring stable transfer and multiplication of transgenes, makes it an ideal candidate for genetic engineering (Azevedo et al. 2011). Genetic transformation holds promise for enhancing economically essential traits in sugarcane and expanding its uses beyond traditional applications like sucrose production. Areas of focus for transgenic sugarcane include improving resistance to insects, diseases, and herbicides; enhancing tolerance to cold, salt, and drought; and boosting sugar and biomass accumulation.

19.8 Conventional Plant Breeding

The intricate genetic composition and absence of resistance genes in the sugarcane genome have made traditional breeding challenging for breeders. Therefore, conventional sugarcane breeding faces limitations due to a narrow gene pool and complex genome originating from a limited set of progenitor clones (Sreenivasan et al. 1987). Additional challenges include infertility, extended breeding cycles, and cold sensitivity, further hindering improvements in certain sugarcane strains. Over the past three to four decades, traditional breeding has successfully enhanced yield, ratooning ability, and pest resistance (Jackson 2005). Conventional breeding approaches primarily rely on empirical selection for yield (Lakshmanan et al. 2006; Azevedo et al. 2011), though this method is suboptimal, as yield is a quantitative trait with low heritability and significant genotype-by-environment (G × E) interactions.

Advances in crop physiology and genomics have provided breeders with valuable insights into stress tolerance and innovative tools for crop improvement (Atlin and Lafitte 2002; Verma et al. 2023a, b; Zheng et al. 2023). Natural selection favors beneficial variation and survival traits while breeding efforts have focused on increasing the economic yield of cultivated species (Tuberosa and Salvi 2006). Through selecting for yield and secondary traits, conventional breeding has addressed genetic improvements in pest resistance (Cattivelli et al. 2008). Advances in molecular techniques have also notably enhanced germplasm utilization and introduced innovative methods for breeding under stress conditions (Azevedo et al. 2011; Farooq et al. 2012). Numerous molecular resources have been developed and explicitly identified for advancing sugarcane, with breeding programs primarily targeting traits that improve resistance (Sreenivasan et al. 1987).

19.9 Tissue Culture Approaches

The initial research on callus induction at the Hawaiian Sugar Planters' Association Experiment Station and the method for root production (Nickell 1964) marked a turning point for sugarcane tissue culture, paving the way for wide-ranging research applications. Later, Heinz and Mee (1996) provided the first insights into sugarcane regeneration. These in vitro methods have significantly impacted both fundamental and commercially driven research, including the conservation of superior germplasm, creation of agronomically improved some clones, large-scale propagation of top-performing clones, generation of pest-free planting material, and testing for resistance to various stresses, including those from biotic factors. In terms of resistance to sucking insects, these methods offer valuable pathways for developing sugarcane lines with enhanced resilience, supporting sustainable crop protection, and reducing reliance on chemical insecticides (Azevedo et al. 2011; Mustafa et al. 2018).

19.10 Breeding Approaches

Breeding strategies to control sucking pests primarily focus on developing crop varieties with inherent resistance mechanisms, achieved through selecting plants with traits like thick leaf cuticles, anti-feeding compounds, and the ability to attract beneficial insects, alongside practices like crop rotation and intercropping to disrupt pest life cycles and minimize reliance on chemical pesticides. Besides, resistance to pest saliva often incorporates genetic diversity from wild sugarcane relatives to enhance pest resistance; this can be achieved through traditional breeding methods or by utilizing genetic engineering techniques to introduce specific resistance genes into elite sugarcane cultivars.

19.10.1 Genetic Engineering Approaches

The development of genetic resistance plays a crucial role in integrated pest management (IPM) by enabling plants to resist a broad spectrum of pests (Francia et al. 2005; Azevedo et al. 2011). Genetic resistance has primarily been observed in crop wild relatives, which were selected during plant domestication and later through plant breeding. Genetic modification using stacked gene technology (combining multiple genes) has been applied to sugarcane by introducing modified cane borer-tolerant (CEMB-Cry1Ac) and glyphosate-tolerant (CEMB-GT) genes (Francia et al. 2005; Verma et al. 2022a, b). Such genomic technology needs to be extended for sucking insects of sugarcane.

19.10.2 Genetics of Resistance

Genetic engineering for insect resistance involves introducing specific DNA sequences into crop plants to improve their ability to resist insect pests (Francia et al. 2005; Deng et al. 2008). These DNA sequences typically encode insecticidal proteins, so plants that incorporate this DNA produce an insecticidal protein (Francia et al. 2005; Deng et al. 2008; Azevedo et al. 2011). Modern biotechnological tools such as genome editing, host-induced gene silencing (HIGS), and CRISPR/Cas9 can be utilized in genetic engineering to achieve sustainable control of insect pests in sugarcane (Verma et al. 2022a, b).

19.10.3 Genome Editing

Gene editing, or genome editing, is a form of genetic engineering that precisely alters a gene's function by inserting, deleting, or replacing DNA bases at a specific target sequence in an organism's genome, using the cell's natural repair processes (Francia et al. 2005; Deng et al. 2008; Azevedo et al. 2011; Bortesi and Fischer 2015; Verma et al. 2022a, b). Due to the sugarcane genome's complexity, only a limited number of reports are available for this crop (Eid et al. 2021; Verma et al. 2022a, b). However, it presents a growing opportunity in insect pest management, expanding the potential to strengthen plant resistance against sucking insects. Sugarcane has a massive genome (~10 GB) that is highly complex, with an auto-allopolyploid structure, limited genetic diversity, and significant recombination between its two sub-genomes (Francia et al. 2005; Eid et al. 2021; Singh et al. 2022; Guo et al. 2023).

Furthermore, it is highly heterozygous and requires lengthy vegetative propagation to maintain genetic consistency. Despite these challenges, genetic transformation and omics approaches have been instrumental in advancing sugarcane improvement efforts (Azevedo et al. 2011; Schaart et al. 2021; Verma et al. 2021a, b; Malviya et al. 2022). Four nuclease families can be employed for genome editing: meganucleases, transcription activator-like effector nucleases (TALENs), zinc-finger nucleases (ZFNs), and clustered regularly interspaced short palindromic repeats (CRISPR)-associated nucleases (CRISPR/Cas9 technologies) (Francia et al. 2005; Deng et al. 2008; Azevedo et al. 2011; Kathiria and Eudes 2014). Notably, the CRISPR/Cas9 genome-editing system is effective for use in sugarcane, even with its high ploidy levels and offers precise mutagenesis capabilities that can be leveraged to enhance resistance against sucking pests (Azevedo et al. 2011; Eid et al. 2021). At present, four primary nuclease-based genome-editing technologies are available: meganucleases, zinc finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs), and CRISPR/Cas9 (Francia et al. 2005; Deng et al. 2008; Eid et al. 2021).

Of these, CRISPR/Cas9 has emerged as a powerful tool for selectively targeting genes involved in pest resistance, including genes that may contribute to sugarcane's susceptibility to sucking insects (Shabbir et al. 2021). The CRISPR/Cas9 system comprises two primary components: a guide RNA and the Cas9 endonuclease (Deng et al. 2008; Azevedo et al. 2011). The guide RNA, a single-stranded molecule, contains a 20-base pair sequence that matches the target region in the host DNA. Cas9 introduces double-stranded breaks (DSBs) three base pairs upstream of the PAM sequence (5′NGG3′) in the DNA, which corresponds to the gRNA sequence (Jinek et al. 2012; Eid et al. 2021). The DNA repair mechanisms of the host cell then fix these breaks, primarily through non-homologous end joining (NHEJ) or homology-directed repair (HDR). NHEJ, which is more common, is prone to errors and frequently leads to mutations through insertions, deletions, or base substitutions (Deng et al. 2008; Azevedo et al. 2011; Jiang et al. 2014).

In addition to Cas9, numerous Cas variants have been identified and employed in genome editing. A challenge with vector-based CRISPR/Cas delivery is that the editing cassette, including markers, promoters, and the Cas9 gene, becomes integrated into the plant genome, transmitting to subsequent generations and thus categorizing genome-edited (GE) plants developed this way as genetically modified organisms (GMOs) (Azevedo et al. 2011; Chapman et al. 2012). Transgenes can sometimes be removed through recombination and selection for seed-propagated annual crops. However, for highly heterozygous, vegetatively propagated crops like sugarcane, removing transgenes through meiotic recombination to generate genetically engineered lines with identical genome composition, except for the targeted region, is a difficult task (Rodgers and McVey 2016; Pannunzio et al. 2018; Verma et al. 2022a, b). The emergence of transgene-free genome-editing techniques, which allow for targeted mutations without residual transgenes, provides valuable methods to develop GE sugarcane without foreign gene integration (Verma et al. 2022a, b). Thus, the concept of genome editing is for creating and screening transgene-free GE sugarcane lines to enable rapid genetic advancements, particularly for enhancing resistance toward biotic stress factors that to significant sucking pests.

19.10.4 Gene Transformation

Genetic transformation in crops for insect resistance involves introducing a particular DNA sequence or gene into plants, allowing them to withstand insect pests (Francia et al. 2005; Deng et al. 2008). The introduced DNA typically encodes an insecticidal protein, which when expressed in the plant, provides targeted resistance against certain insect pests (Deng et al. 2008; Azevedo et al. 2011; Gatehouse 2013). This method has been used to address a range of insect pests, particularly those from the Hemiptera order (Birkett and Pickett 2014). In the field of gene transformation, several strategies are being explored to protect plants from insect damage. Lectins, naturally found in many plants, bind to carbohydrates in the midgut of herbivorous

insects, interfering with their digestion (Francia et al. 2005; Vandenborre et al. 2011). Transgenic approaches have incorporated protease inhibitors in plants, hindering insects' ability to process their food (Azevedo et al. 2011; Singh et al. 2020a, b; Sharma et al. 2021; Li et al. 2023).

Additionally, transgenic plants expressing alpha-amylase inhibitors have been developed to provide resistance against different pests; these inhibitors, once cloned into plants, exhibit effective insecticidal properties against Hemipteran pests. Insect chitinase and chitinase-like proteins also play a crucial role by breaking down chitin in the insect exoskeletons and gut linings, further enhancing plant resistance (Francia et al. 2005; Deng et al. 2008). The soil bacterium *Bacillus thuringiensis* (Bt) is a gram-positive, spore-forming bacterium that produces insecticidal proteins toxic to many insects. The combined application of *Bt* and *Trichoderma* can reduce both chewing and sucking insect pests. The vegetative insecticidal proteins (Vip) family of *Bt* proteins is well-regarded for its insecticidal effectiveness produced during the plant's vegetative growth phase (Deng et al. 2008). Vip proteins hold promise due to their unique sequence homology and distinct receptor binding sites compared to Cry proteins. They are classified into three subfamilies: Vip1, Vip2, and Vip3, which form heterodimeric toxins. Vip1, in particular, targets the Hemiptera order, which includes major sucking pests of sugarcane. Vip proteins can be used alone or in combination with Cry proteins to improve pest control effectiveness (Deng et al. 2008; Gupta et al. 2021).

Plant lectins function as storage proteins and play a role in defending against phytophagous insects. Several plant-derived lectins have been identified as toxic to important insect species within the Homoptera order (Powell et al. 1993; Sauvion et al. 1996; Francia et al. 2005). Transgenic rice plants, modified to express the insecticidal protein snowdrop lectin (GNA) through particle bombardment, showed significant resistance to sap-sucking pests (Sudhakar et al. 1998; Li-Hong et al. 2006). The expression of the GNA gene from *Galanthus nivalis* also provided resistance to aphids in potatoes (Li-Hong et al. 2006; Deng et al. 2008; Mi et al. 2017). Transgenic cotton lines expressing an insect gut-binding lectin demonstrated significant resistance to sucking insects in the T1 generation (Vanti et al. 2018).

Later studies on transgenic sugarcane lines, engineered with lectin expression driven by either the phloem-specific RSs-1 (rice sucrose synthase-1) promoter or the maize ubi-1 promoter through Agrobacterium-mediated transformation, showed marked reductions in the fecundity and survival of *Ceratovacuna lanigera* in sugarcane. These modifications slowed the insect's development and deterred feeding. Aphid populations on these transgenic plants were reduced by 60–80%, with some lines showing reductions as high as 95% (Zhangsun et al. 2007; Deng et al. 2008). The most significant resistance observed with the RSs-1 promoter suggests that it is especially effective in modifying plants to resist sap-feeding pests such as woolly aphids, as it facilitates phloem-specific expression of the transgene (Zhangsun et al. 2007; Deng et al. 2008).

19.10.5 Marker-Assisted Selection

Marker-assisted selection (MAS) uses molecular markers to facilitate phenotypic selection in crop improvement (Francia et al. 2005; Li-Hong et al. 2006; Deng et al. 2008). This approach involves selecting individuals based on their genetic marker profiles (genotype) rather than their observable characteristics (phenotype). Several types of molecular markers, such as single nucleotide polymorphisms (SNPs), have been identified and hold great potential for enhancing the efficiency and precision of traditional plant breeding (Francia et al. 2005; Li-Hong et al. 2006; Deng et al. 2008). Molecular markers and small DNA sequence fragments display polymorphism, highlighting variations across different organisms. Various types of molecular markers, including AFLPs (Amplified Fragment Length Polymorphisms), RAPDs (Random Amplified Polymorphic DNA), SSRs (Simple Sequence Repeats), RFLPs (Restriction Fragment Length Polymorphisms), TRAPs (Target Region Amplification Polymorphisms), SNPs (Single Nucleotide Polymorphisms), and ISSRs (Inter Simple Sequence Repeats), have been extensively applied in sugarcane genetic research (Li-Hong et al. 2006; Deng et al. 2008; Ali et al. 2019; Manimekalai et al. 2020).

Molecular marker techniques are the most advanced methods for introducing targeted genes into crop plants in precise combinations. They are extensively used and offer a new opportunity to improve crop yields (Das et al. 2017). For instance, the rice variety ASD 7, which contains the BPH resistance gene bph2, was crossed with the susceptible cultivar C418, a japonica restorer line, and evaluated using marker-assisted selection (MAS) (Li-Hong et al. 2006; Deng et al. 2008). This cross demonstrated significantly improved resistance to the brown plant hopper, *Nilaparvata lugens*, a significant pest of rice crops (Francia et al. 2005; Li-Hong et al. 2006). Shabanimofrad et al. (2015) also developed rice varieties using MAS that resisted biotypes 2 and 3 of the brown planthopper.

19.10.6 Insect Resistance Mediated by R-Genes

R genes, or resistance genes, are genes in plant genomes that produce R proteins that help plants resist disease (Francia et al. 2005; Deng et al. 2008). While some resistances protect a wide range of pest species, most are specific to particular herbivore species (Azevedo et al. 2011). Natural resistances, often found in wild relatives that can be crossbred with current crops, are particularly effective against pest species that target specific plant organs or tissues, such as aphids, whiteflies, and other phloem-feeding insects. R-gene-mediated resistance is a plant defense mechanism (Fig. 19.3) that protects plants from sap-sucking pests through a "gene-for-gene" interaction. In this process, the plant specifically detects a substance secreted by the insect, triggering a defense response.

Only a limited number of dominant R-genes that confer resistance to phloem-feeding pests, such as Mi-1.2, VAT, and BPH16, have been cloned. However, many

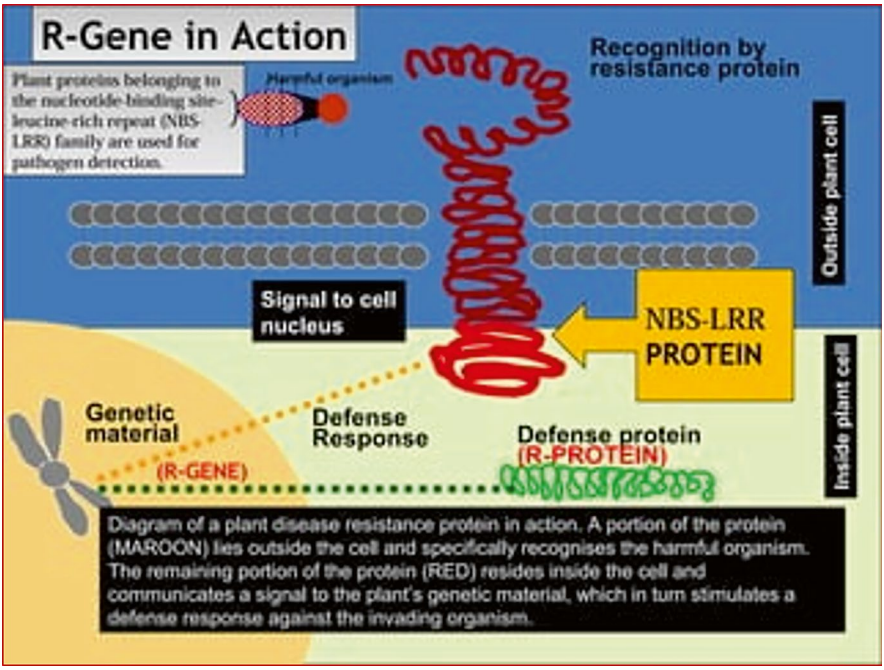

Fig. 19.3 R-gene-mediated resistance is a plant defense mechanism

more of these genes are widely utilized in agricultural practices through marker-assisted breeding (Francia et al. 2005; Deng et al. 2008; Azevedo et al. 2011). Hence, R-gene-mediated insect resistance found in nature is most effective against pests, such as phloem-feeding insects, that rely on close interaction with their host plant for successful colonization. These pests are more likely to be controlled through R-gene-mediated defenses (Francia et al. 2005; Deng et al. 2008; Azevedo et al. 2011). Interestingly, the exact mechanism of action for these resistances remains unclear even in crops where the R-gene has been cloned and characterized. It likely involves the recognition of the attacker followed by downstream signal transduction, which triggers a defense response that prevents phloem-feeding insects from sustaining prolonged feeding.

19.10.7 RNAi Approach

RNA interference (RNAi) is a promising approach for improving insect resistance in sugarcane and other crops. RNAi is a naturally occurring process that silences genes in a sequence-specific manner (Francia et al. 2005; Hellmich et al. 2008; Deng et al. 2008; Singh et al. 2023). RNAi-based biopesticides can be used to target genes in insects, weeds, and agricultural diseases, which can hinder their growth and reduce their damaging effects on crops. In insect-consumed plant tissues,

double-stranded RNAs targeting critical insect genes can be consistently expressed (Francia et al. 2005; Deng et al. 2008; Azevedo et al. 2011). This activates the RNA interference (RNAi) pathway, leading to the degradation of mRNAs from these essential genes (Hellmich et al. 2008; Price and Gatehouse 2008; Agarwal et al. 2012; Gupta et al. 2021; Malviya et al. 2024). RNAi targets have been identified for several crucial insect genes, including those encoding developmental proteins, digestive enzymes, salivary gland proteins, regulatory proteins in the nervous system, proteins involved in host-insect interactions, hormone receptors, gut enzymes, and metabolism-related proteins (Francia et al. 2005; Deng et al. 2008; Gatehouse 2008; Hellmich et al. 2008; Huvenne and Smagghe 2010; Kalunke et al. 2009; Gupta et al. 2021).

The RNA interference (RNAi) strategy is a homology-dependent gene silencing mechanism that involves mRNA degradation, and it is a pathway found in all eukaryotic organisms. Li et al. (2015) found that double-stranded RNAs (dsRNAs) remain stable in various conditions and can be absorbed through the roots of crop plants. This discovery suggests that dsRNAs could be utilized as biopesticides. RNA interference (RNAi) is an effective method for managing insect pests (Francia et al. 2005; Deng et al. 2008; Huvenne and Smagghe 2010; Azevedo et al. 2011). When insects consume plant material containing dsRNA, it enters their gut, triggering the RNAi process and silencing specific target genes in the pest (Gatehouse 2013; Gupta et al. 2021). For RNAi-based control of insect pests to be successful, it is crucial to accurately identify specific insect enzymes and proteins as targets, develop effective methods for introducing dsRNA into insect cells or bodies, and ensure the stability of dsRNA both during and after its delivery (Francia et al. 2005; Deng et al. 2008; Gatehouse 2013; Gupta et al. 2021). Additionally, combining RNAi technology with *Bt* or other approaches provides a valuable strategy for managing insect pests that may develop resistance to insecticidal proteins (Gatehouse 2013; Gupta et al. 2021; Padmavathi and Padmaja 2022).

19.10.8 MicroRNA Approach

MicroRNAs (miRNAs) are small, non-coding RNA molecules that play a role in regulating gene expression in both plants and insects, and they can be utilized to enhance plant resistance to insects (Francia et al. 2005; Deng et al. 2008; Hellmich et al. 2008; Kalunke et al. 2009; Gatehouse 2013; Gupta et al. 2021). In contemporary plant protection strategies, miRNA spray technologies are promising for boosting plant resistance to pests. miRNAs are small, non-coding RNA molecules that regulate gene expression and are crucial in plant defense processes (Kalunke et al. 2009). Over the past two decades, regulating cell growth and development, gene translational repression, and stress responses through microRNA (miRNA) mechanisms have been thoroughly studied and evaluated (Hellmich et al. 2008; Gupta et al. 2021). Recent studies suggest that miRNAs play a crucial role in regulating

biological processes during plant–insect interactions, contributing to enhanced pest resistance (Francia et al. 2005; Deng et al. 2008; Hellmich et al. 2008; Kalunke et al. 2009; Gatehouse 2013). Numerous miRNAs have been identified in various crop plants that respond to and protect against insect attacks (Gatehouse 2013). Moreover, cross-kingdom RNA interference mediated by miRNAs plays a significant role in plant-insect interactions (Gupta et al. 2021). Furthermore, several miRNA-based approaches are currently being utilized to improve field crops.

19.11 Conclusions

Sugarcane varieties are fundamental to the long-term sustainability of cane cultivation. Developing new varieties continues to be a top priority for stakeholders in the global sugarcane industry. The advancements in sugarcane breeding, combining traditional, molecular, and biotechnological techniques, have made significant progress in improving resistance against sucking insect pests. In addition, although conventional breeding has been essential for improving yields and ratooning capacity, the incorporation of molecular markers, genetic transformation, and genome editing techniques like the CRISPR-Cas9 approach has opened new pathways for developing pest-resistant varieties with targeted desirable traits. Genetic engineering approaches, including RNAi, miRNAs, and R-genes-mediated resistance to insects**,** further contribute to constructing precise pest control strategies, reducing overdependency on chemical pesticides. These innovations collectively represent a more sustainable path forward in sugarcane production, addressing challenges in sucking pest management of sugarcane with improved crop resilience. The genetic enhancement of sugarcane through traditional breeding is progressively supported by molecular research and genomic engineering to boost natural resistance, which can play a vital role in reducing the effects of biotic and abiotic stressors on future sugarcane farming systems. Future studies should aim to refine these methods to ensure their effectiveness in addressing the challenges posed by the changing climate and evolving pest dynamics, ultimately enhancing the sustainability of sugarcane cultivation.

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Chapter 20
Sugarcane Smut Disease Resistance: Challenges to Sugarcane Breeding in China

Smita Lata, Shruti Kumari, Sumira Malik, Jutishna Bora, Ajay Kumar Mahalka, Sarvesh Rustagi, Bipin K. Sati, Seema Ramniwas, and Xiu-Peng Song

Abstract The fungal species *Sporisorium scitamineum* causes sugarcane smut and is a severe pathogen in China that imposes substantial yield losses on sugar production throughout the country. As this disease is one of the main diseases found in most places cultivating sugarcane, namely in Guangxi, Yunnan, and Guangdong, it becomes very challenging for breeders who want to breed disease-resistant varieties. Sugarcane is complex at the genetic level, and the pathogen has an adaptive ability that makes it challenging to breed for durable resistance. Although traditional breeding techniques are fundamental, they can never keep pace with the rate of adaptation of the pathogen. Recent developments in molecular breeding, such as MAS, genomics, and transgenic approaches, are promising tools for enhancing resistance efficiency. However, such breeding techniques are hard to apply in large-scale breeding programs since the crop has a long breeding cycle, and variability is high with a demand for improvement of multiple traits. The paper outlines the cur-

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rent status of sugarcane smut disease in China. It discusses developing disease-resistant varieties and possible solutions to this ravaging disease through newly developed technology. Recent developments in omics technologies, transcriptomics, proteomics, and metabolomics contribute to our understanding and insight into resistance and susceptibility mechanisms. This will further our knowledge about smut and its interaction with sugarcane genotypes and thus support the development of durable, resistant varieties for sustainable sugarcane in years to come.

Keywords Disease tolerance capacity · *Saccharum* spp. · *Sporisorium scitamineum* · Sugarcane productivity · Unfavorable growth conditions · Environmental Sustainability · Molecular breeding strategies

20.1 Introduction

Sugarcane smut disease, caused by the fungus *Sporisorium scitamineum,* is a significant threat to sugarcane production worldwide, including in China. This disease can lead to severe yield losses, reducing both the quantity and quality of sugarcane produced. Smut disease is characterized by the formation of whip-like structures, stunted growth, and reduced cane yield, ultimately impacting sugar output (Albert and Schenck 1996; Singh et al. 2019). In China, sugarcane is an important economic crop, particularly in southern provinces such as Guangxi, Yunnan, and Guangdong (Mo 2012; Song et al. 2019). It supports local economies and provides raw materials for the sugar industry (Verma et al. 2020a, b). However, sugarcane smut disease has posed persistent challenges to breeders aiming to develop resistant varieties. Although some varieties exhibit partial resistance, the fungus's ability to mutate and overcome these resistance mechanisms has complicated breeding efforts (Li et al. 2012; Afghan et al. 2024). Developing resistant varieties through conventional breeding methods is time-consuming and labor-intensive due to the long growth cycle of sugarcane. Furthermore, sugarcane's polyploid nature and genetic complexity complicate the identification of resistant genes. Breeders face the additional challenge of balancing smut resistance with desirable traits, such as high yield, sugar content, and adaptability to local environments (Benda and Koike 1985; Verma et al. 2024a, b).

Despite these challenges, advancements in molecular breeding, marker-assisted selection, and biotechnology offer promising avenues for improving sugarcane resistance to smut disease in China. However, integrating these techniques into mainstream breeding programs remains formidable, requiring coordinated efforts between researchers, breeders, and industry stakeholders (Chao et al. 1989; Song et al. 2021). In summary, sugarcane smut disease continues to pose a significant challenge for sugarcane breeding in China. Efforts to develop resistant varieties must be strengthened to ensure the sustainable production of this economically substantial crop (Singh et al. 2021a, b).

20.2 Impact on the Sugar Industry

The spread of sugarcane smut in China significantly impacted the sugar industry, especially when the disease peaked in the twentieth century. The outbreak led to a sharp decline in yield and quality, resulting in financial losses to the farmers and the sugar mills, which needed a continuous supply of quality cane (Li et al. 2024; Chen et al. 2023, 2024). The disease also increased the cost of production because farmers had to spend money on disease control measures and replant using resistant varieties (Li et al. 2023). China's sugar industry has reacted to the situation by modernizing its approach to crop management and investing in research and development (Verma et al. 2023a, b). Such practices as breeding for disease resistance, improved farming, and clean planting material have stabilized sugar production against sugarcane smut. As a result, the history and spread of sugarcane smut in China have depicted the problems that plant diseases have posed in large-scale agriculture (Guo et al. 2022, 2023; Tian et al. 2023).

It was a devastating disease to the extent it spread. However, what China has managed by breeding, improving agricultural techniques, and training farmers has lessened the disease's adverse effects. Still, vigilance is required to prevent new strains from undermining the development made so far (Dean 1982; Liang et al. 2024).Four weeks following wound inoculation, the meristematic region was stained with trypan blue to demonstrate *S. scitamineum* colonization: A, a highly susceptible genotype exhibiting a high density of mycelium concentrated at the internodes; B, a highly resistant genotype exhibiting no mycelium; and C, mycelium moving along the xylem vessels within a susceptible genotype (Nallathambi et al. 1998).

20.3 Sugarcane Smut Disease Overview

20.3.1 Pathogen Description / Causative Agent

This sugarcane smut disease is caused by the basidiomycete fungus, previously known as *Ustilagoscitaminea*. Over time, it has been thought to be one of the most important diseases of sugarcane worldwide and in China. This pathogen primarily infects the growing points of the sugarcane, thereby producing whip-like structures characteristic of this disease. It can spread rapidly throughout the sugarcane fields, making many teliospores resistant to environmental conditions and remaining in the soil for some years. These spores are spread by the wind or by human activities, such as machinery and tools used inside the field, and are very efficient in the speedy spread of the disease. Once *S. scitamineum* infects the sugarcane plant, it colonizes the meristematic tissues, halting its expected growth. Eventually, the fungus drains the resources of the plant, thus depleting its vigor and productivity (Rott et al. 2000).

The genetic variability of the pathogen, with its ability to mutate and adapt, has made smut disease a persistent and ever-changing challenge for sugarcane farmers, which, in turn, calls for continuous efforts to breed resistant varieties. Fungal Structure: This fungus has produced black, smut-like galls that contain masses of spores. Such galls can develop at any part of the sugarcane plant, especially on the stalks. Lifecycle: This fungus follows a complex lifecycle encompassing sexual and asexual reproduction. It disperses its spores through the wind, rain, and mechanical means. Pathogenicity infects the sugarcane plant, causing abnormal growth and gall formation. This disrupts normal plant development and can substantially decrease yield and sugar content (Burner et al. 1993).

20.3.2 Symptoms and Impact

The symptoms of sugarcane smut disease are relatively easy to recognize and frequently catastrophic for affected crops. The most characteristic symptom is the appearance of a black, whip-like structure, called a "smut whip," which grows out of the spindle or topmost part of the infected plant represented in (Fig. 20.1). The spore-infested whip that grows on sugarcane worsens the infection through contact with surrounding plants. Infected plants exhibit short, tillered plants with decreased vigor. Leaves of the affected plants can become flimsy, and the overall aspect of the crop is drastically reduced, so many of the plants will not be fully grown to their size or reach their normal maturation stages. This tends to result in a considerable reduction in sugar content and overall biomass because the pathogen siphons off energy from the plant from the production of sugars to the maintenance of the fungal infection (Chassot et al. 2007).

Control Measures-Resistant Varieties: Using disease-resistant sugarcane varieties is one of the most effective control methods. Cultural Practices: Implementing good agricultural practices, such as crop rotation and proper sanitation, can help minimize the spread. Chemical Control: Fungicides may be used in some cases, though their effectiveness can be limited. The economic losses of sugarcane smut disease in China are simply too high. The infection lowers the yield and quality of the harvested sugarcane, which means reduced sugar per hectare. Infected plants produce less sugar and are more susceptible to secondary infections and other environmental stresses, such as drought or nutrient deficiencies, which cause additional damage (Chen et al. 2020; Huang et al. 2022; Oliveira et al. 2022). Severe outbreaks of smut can destroy sugarcane crops, and farmers may opt to destroy infected crops and replant with resistant varieties- a costly and time-consuming process. It is, therefore, directly impactful on the profitability of sugarcane farming and has the potential to threaten not only the small-scale farmers but also the more significant sugar industry in China (Dean 1982).

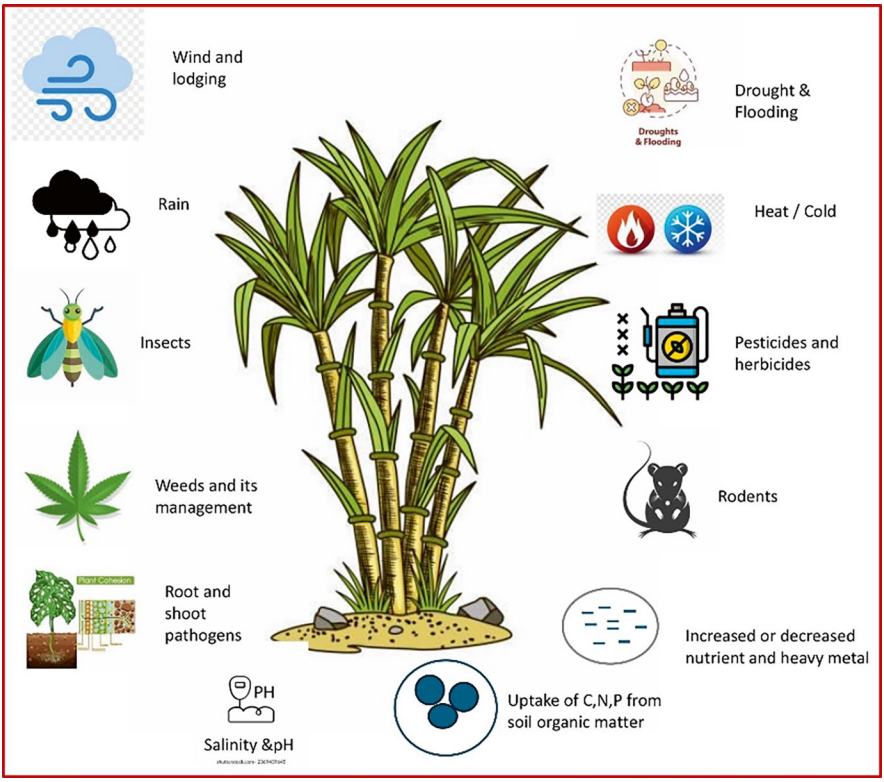

Fig. 20.1 Various abiotic and biotic stress affect sugarcane plant growth, development, and productivity

20.4 Geographic Distribution in China / Quality and Yield Production

The disease is prevalent in most sugarcane-growing regions of China, particularly in the southern provinces whose climatic conditions are very favorable for the growth of the pathogen. The principal provinces of sugarcane in the country include Guangxi, Yunnan, and Guangdong, and this has been the most significant place affected by sporadic outbreaks of smut disease (Chen 2012). The warm and moist weather of southern China presents excellent conditions for spores of *S. scitamineum* to increase and develop. High temperatures and continuous rain have generally favored the disease in an area where spores tend to germinate and cause infection (Singh et al. 2019). Despite introducing resistant varieties, the outbreaks of this disease still afflict these critical regions (Gong et al. 1996). The geographical spread of sugarcane smut in China has dramatically influenced the quality and yield of production. The yield per hectare has fallen by massive proportions in the highly infested areas. The quality of sugarcane, measured by the sucrose content in the

harvested stalks, also deteriorates as the disease progresses. Plants weakened by the infection cannot accumulate sugars effectively, leading to low-quality crops that fetch reduced prices in the market.

The disease increases production costs because investment in smut-resistant varieties and stringent sanitation measures are needed, and sometimes chemical treatments to control the pathogen have to be applied. Although resistant varieties were developed and deployed throughout sugarcane-growing areas in China, the constant evolution of *S. scitamineum* and environmental conditions favorable to its spread demand further vigilance as well as innovation in breeding and disease management strategies. Sugarcane smut disease is an essential threat to the Chinese sugarcane industry, and its effects can be noticed on yield and quality in major production regions (Verma et al. 2019a, b; Song et al. 2022; Zheng et al. 2023). In this context, because sugarcane is an important crop in China's domestic economy, especially for the sugar and ethanol industries (Verma et al. 2021a, b; Huang et al. 2024), addressing the issue of smut disease using resistant varieties and sustainable management practices is important in maintaining productivity and economic stability (Singh et al. 2020; Xu et al. 2022). Minimizing the spread of sugarcane smut in Chinese sugarcane farming will be contingent upon future breeding programs and integrated disease management strategies (Que et al. 2004; Singh et al. 2022). Production of sugarcane from 2010 to 2019 in China (Fig. 20.2).

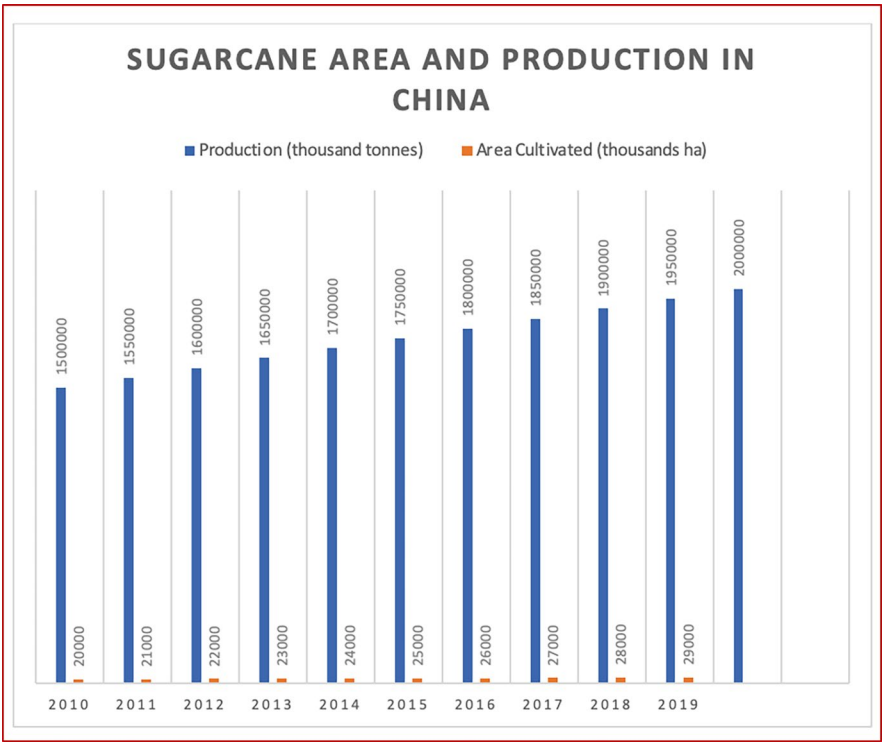

Fig. 20.2 Sugarcane acreage and production from 2010 to 2019

20.5 Sugarcane Breeding and Genetic Resistance

Breeding for sugarcane is through the traditional method of finding a parent plant with specific characteristics, such as higher sugar content, resistance to disease, or drought tolerance. Cross-pollination produces offspring with improved traits. It begins with the discovery of potential parent plants that already exist in the available varieties or wild relatives of sugarcane. They are then crossed through controlled pollination to combine the genetic material of the parents. These seeds grow into seedlings tried out over many years to exhibit the needed characteristics. Plants with more hope of producing are put out into field tests under many environmental conditions to observe whether the plants can work uniformly through various climatic regions and areas (Xu and Chen 2001; Wu et al. 2022a, b). Traditional breeding depends on the available variation within a species. A cycle may take two to three years. This process is lengthy because the growth cycles of sugarcane are very long, and the process requires heavy field testing.

Additionally, conventional breeding methods can mostly not introduce new characteristics, particularly those not found within the sugarcane gene pool. To overcome the challenges in the above list, most breeders have adopted highly modern techniques such as markers and molecular breeding to do precise selection and quick variety production. However, traditional breeding remains the bottom line in sugarcane improvement, especially in creating and maintaining genetic diversity so that newly emerging pests or diseases can be effectively eliminated. "Genetic resistance" in the sugarcane refers to the innate characteristics of a sugarcane plant (Que et al. 2011; Verma et al. 2020c, d). Such a plant is born with an inherent ability not to get infected by various pathogens such as viruses, fungi, and bacteria. Of course, the degree to which a given variety of sugarcane possesses resistance against infection is variable; there are key points here: innate immunity is essential for most plants and is formed by the structural and biochemical barriers preventing infection. Resistant Germplasm: This relates to the breeding and selection of sugarcane varieties with inherent resistance to certain diseases. Germplasm that harbors resistance traits is critical to breeding programs to develop disease-resistant sugarcane varieties. These traits are often discovered through traditional breeding and modern biotechnological approaches (Whittle and Walker 1982; Joshi et al. 2022).

Molecular Mechanisms: Most resistances involve more than one gene. Advanced genetic engineering techniques, such as CRISPR and gene cloning, can enhance or introduce the resistance traits from resistant germplasm into high-yielding but susceptible sugarcane varieties (Su et al. 2013). Role of Biotechnology: Utilization of improved biotechnological tools helped in the discovery of genes associated with resistance to be incorporated and used to generate novel versions with precise breeding methodologies that have used marker-assisted selection and genomic selection during the process of cultivar development for enhanced disease resistance (Xu and Chen 2000). The "Resistant Germplasm" section in the paper may take care of how these resistant varieties are isolated, bred, and incorporated into agriculture to manage diseases and their impact on sugar production (Fig. 20.3).

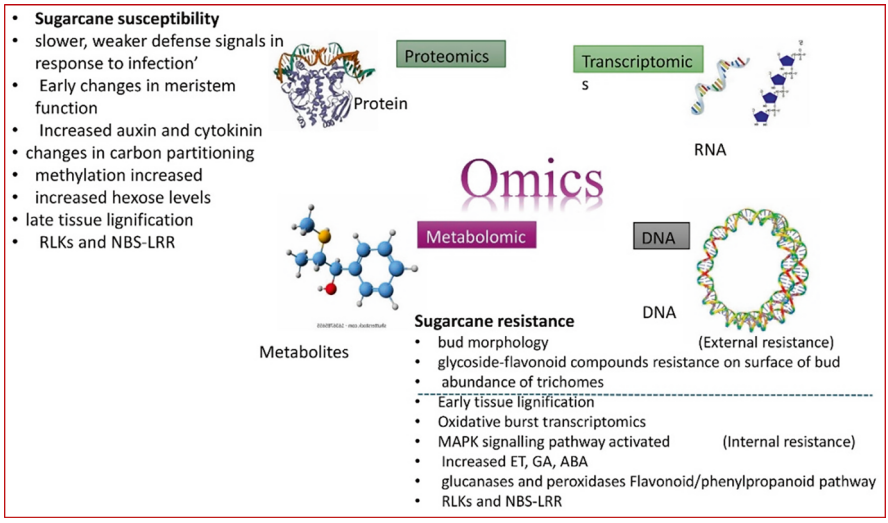

Fig. 20.3 Figure depicting the differential responses to *Sporisorium scitamineum* infection in resistant and susceptible sugarcane plants via omic approaches

20.6 Breeding Challenges in China

It has unique challenges in breeding because the country has diversified climatic conditions, growing circumstances are quite complex, and the crop biologically is complex. Sugarcane is the staple crop in the southern regions of China, predominantly grown in Guangxi, Yunnan, and Guangdong (Malviya et al. 2020, 2022; Guo et al. 2021; Verma et al. 2023c, d). Integrating resistance traits without negatively affecting other desirable agronomic traits, such as yield, sugar content, and growth rate, is still challenging. Introduced resistance must be durable and effective against more than one strain of the same pathogen that could evolve over time. However, these provinces are suitable for plant growth, but specific challenges also arise for breeding programs requiring higher yields, resistance, and adaptability (Shen et al. 2016; Singh et al. 2023; Bhatt et al. 2024).

20.7 Diverse Climatic Conditions and Growing Environments

The sugarcane-growing regions in China experience highly varied climatic conditions ranging from tropical to subtropical climates (Verma et al. 2020e, f, 2022a, b), which can make breeding varieties that could thrive in all these variable environments difficult. The varying rainfall patterns, temperatures, and soil types necessitate this diversity in the breeding programs (Sharma et al. 2021, 2022). For instance, sugarcane cultivated in the southern provinces of Guangxi and Guangdong might be

subject to intense rainfall and humidity (Singh et al. 2021; Deng et al. 2023). At the same time, the risk of fungal diseases like smut or rust is high. Conversely, aridity and coolness in the province of Yunnan introduce drought and lower temperature stress factors that need stress-tolerant varieties by drought resistance and cold hardiness. An overall challenge is to create one variety that can do well under all these stresses. Most region-specific breeding activity must be implemented (Xu et al. 1994).

20.7.1 Pest and Disease Pressures

Different pests and diseases in other areas of China attack the sugarcane. Generally, this is the single major threat that sugarcane encounters. The most prominent sugarcane diseases across sugarcane farms are SCMV, smut, rust, and red rot. These diseases mainly affect crop yield and the quality of sugarcane crops in the country. Breeding for resistance to such diseases becomes complicated because sugarcane is a complex polyploid plant with many chromosomes. In this case, controlling the inheritance of specific resistance traits is challenging. More importantly, the pests—the borers and the termites—can cause tremendous damage to the crop, hence requiring breeders to develop disease-resistant varieties that could withstand pest infestations (Wen et al. 2006). In addition, the problem of newly emerging races and changing pathogens is seen. For instance, a new virulent race emerges in sugarcane smut; hence, breeding efforts must be continually made to keep up with such evolving threats. Mechanisms against pest resistance, such as that for borers, must also be incorporated into the crop without compromising other desirable traits like sugar content or biomass yield.

20.8 Genetic Complexity of Sugarcane

Sugarcane is a genetically complex polyploid crop, which means its chromosome number is high. Chromosomes are quite large, as in sugarcane, with numbers varying from 100 to 130. As such, traditional breeding is rigid and slow because the sizeable genome renders the conventional system cumbersome due to numerous interactions between many gene copies, which may work unpredictably, making control difficult over the inheritance of the desirable characters or traits. This is more challenging in China because the breeding programs here are limited by access to advanced genetic tools and technologies compared to other countries, which have more advanced sugarcane breeding programs (Que et al. 2011). High genetic complexity also affects the pace of breeding programs. With classical breeding methods, which are crossbreeding and progeny testing, one does not obtain stable varieties. Results often vary as unstable varieties produce more vigorous progenies than parents when released and distributed. For example, only after decades of judicious crossbreeding and selection can one create the ideal variety

that maximally expresses all desirable characteristics, such as sugar content, resistance to some important diseases, and adaptation to local climatic conditions (Schenck 1998).

20.8.1 Limited Germplasm Resources

Breeding programs in China also face a significant challenge: available diversity in sugarcane germplasm. Germplasm refers to the material available for breeding new varieties; limited diversity in such pools will severely limit opportunities to create high-yielding, resilient varieties adapted to local stresses. So far, very limited germplasm from sugarcane breeding program use has been seen in China. This makes a narrow selection of new varieties available because most of the cultivated varieties in China are produced by other countries whose specific priority might not align with China's (Whittle and Walker 1982). There are also challenges when genetic diversity is sought to be introduced by importing foreign germplasm. Importing varieties from other parts of the world often necessitates considerable adaptation to local growing conditions, and certain varieties do not perform as well under the unique conditions prevailing in the sugarcane-growing regions of China (Afghan et al. 2024). Furthermore, the complexity of the genetics of the crop makes the integration of new germplasm slow and uncertain in breeding programs.

20.9 Economic and Technological Constraints

The Chinese sugarcane breeding programs also lack intensive economics and technology. On the contrary, compared to China, the leading sugar producers invest much more resources in research, especially sugarcane breeding. Their economics restrain advanced methods like MAS or genomic selection, which can make breeding very speedy and accurate. Further, competition from other crops and economic sectors hinders the availability of resources for sugarcane improvement (Anas et al. 2021a, b; Malviya et al. 2024). Another challenge to mechanization is the introduction of new problems in breeding for increased efficiency. The plant structure and robustness must change to withstand mechanical harvesting, adding another complexity to breeding goals (Padmanaban et al. 1998).

20.9.1 Breeding for Environmental Sustainability

As the concerns over climate change and environmental sustainability continue to grow, breeding programs for sugarcane in China also need to work on the development of low-input varieties that are low in water, fertilizers, and pesticides. This is particularly challenging since sugarcane is a very water-intensive crop, and many

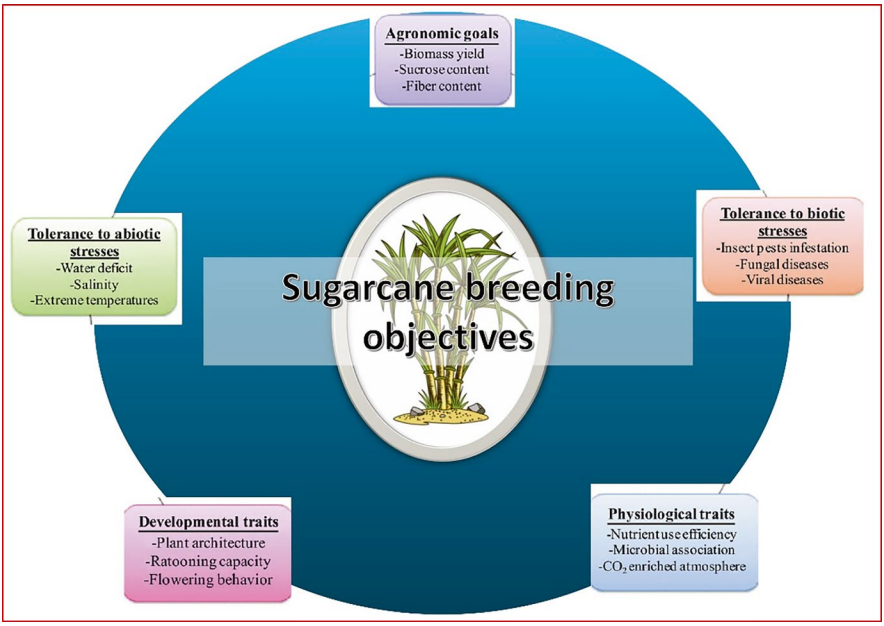

Fig. 20.4 Sugarcane breeding objectives for introgression of desirable traits

regions in China are experiencing water scarcity in the cultivation of sugarcane (Verma et al. 2021c, d, 2022c). Breeding on drought-tolerant varieties comes at the cost of a yield or sugar content; it's a tightrope to walk. Third, with efforts to decrease the environmental impact of China, sugarcane breeders need varieties that are more efficient in the uptake of nutrients, thereby necessitating less chemical fertilizer and reducing the environmental costs of growing sugarcane (Gong et al. 1996). Sugarcane breeding in China has unique challenges based on the diverse climates of its regions, pest and disease pressures, and the genetic complexity of the crop, germplasm resources, and economic and technological constraints. Sustainable investment in research and development in environmentally sustainable high-yield varieties is bound to go a long way in beating these challenges (Fig. 20.4).

20.10 Role of Polyploidy in Sugarcane

Polyploidy refers to a condition where an organism has multiple sets of chromosomes. This important feature in plant biology increases genetic diversity and adaptability. It is significant in sugarcane as it has major implications on disease resistance and the molecular mechanisms involved. Increased Genetic Variation: Increased genomic diversity polyploidy in sugarcane produces multiple gene copies. Consequently, a more excellent reservoir of genetic variation is a result. Therefore, that reservoir may harbor resistance genes or traits toward various pathogens very

well. Independent mutation of the different copies of those genes may arm the sugarcane with some sort of toolkit that gives it more ability to be responsive in the face of a spectrum of stresses ranging from diseases caused by those pathogens to abiotic challenges (Karen et al. 2013).

Gene Redundancy: With multiple copies of genes, the loss or malfunctioning of one copy does not result in the loss of the function because other copies may be able to carry out the essential functions. This redundancy in vital physiological processes may help stabilize even in the face of pathogens and the immune response processes. Enhanced Gene Expression: This can alter the gene expression pattern in the plants, thus enabling them to synthesize more robust and efficient defensive compounds or turn on other immune responses (Bhuiyan et al. 2021). Hybrid Vigor: The breeding stock of sugarcane mainly comes from a different genotype, which is polyploid, and it has the potential to create hybrid vigor or heterosis. It makes the offspring sturdier, grows quicker, and is often resistant to diseases than the parents through gene combination and expression, which come from other pools of genes.

20.10.1 Molecular Mechanisms of Disease Resistance in Polyploid Sugarcane

Gene Silencing and Activation: Polyploidy could influence gene expression in several ways, including through mechanisms of gene silencing or differential gene activation that increase the expression of resistance genes when pathogens are around. Pathogen Recognition Receptors (PRRs): Sugarcane and other plants have several PRRs that detect PAMPs. Polyploidy may influence such receptors' diversity and effectiveness, leading to a more robust recognition system (Dalvi et al. 2012). Signal Transduction: A complex signal transduction cascade triggers the defense machinery upon recognizing pathogens. Therefore, an increase in the complexity and redundancy of such pathways may make plant defenses more effective through polyploidy.

Defensive Compound Synthesis: The synthesis of antimicrobial compounds or cell wall reinforcement is a common approach for resisting pathogens. In some cases, the intensification or diversification of genes for such compound synthesis can be enhanced through polyploidy (Xu and Chen 2000). This is an area of critical research in understanding the role of polyploidy in improving resistance to disease in sugarcane, especially as new varieties are bred to counter the changing landscapes of pathogens with minimal yield or sugar-quality loss. It must intersect with advanced genetic and genomic research to understand multiple gene copies' interactions and how these can be manipulated to improve crop resilience and productivity.

20.10.2 Developing Resistance Varieties

Developing resistant sugarcane varieties against smut disease is complex and involves genetic, environmental, and management factors. This process is very time-consuming and resource-intensive, posing several constraints, and considerations while developing and deploying the resistant varieties.

20.10.3 Environmental Factors

Environmental factors are highly crucial in the development of the disease-resistant sugarcane variety. Local climatic factors, including temperature, humidity, and rainfall, significantly impact the interaction between the smut pathogen and the sugarcane plant (Verma et al. 2024c). In this regard, for example, infection rates increase at higher humidity levels and temperatures because of spores' favored growth and spread. Soil factors, including type and health, also impact vigor in sugarcane by supporting better overall plant health (Verma et al. 2021e, f), and resilience against diseases; well-drained fertile soils are typically the best condition. Crop density and canopy structure inside the sugarcane microenvironment can influence the formation of dew (Joshi et al. 2023), sunlight penetration, and air circulation microclimate around the plants, which can either hinder or promote the growth of the pathogens. Thus, under these environmental conditions, breeding for resistant varieties has to consider all such factors so that the resistance traits work at the specific conditions under which the crop is grown (Wu et al. 2022a, b). In addition, drought and salinity stresses affect the plant's natural resistance mechanism (Verma et al. 2022d, e). Stress conditions weaken the defense system of the plant, thus making it more susceptible to infections. Most breeding programs need to attain improvements in disease resistance and the ability of the plant to resist environmental stresses.

20.10.4 Timeframe and Constraints

The development time for resistant sugarcane varieties can be rather long. Traditional breeding, involving crossbreeding selected varieties that have already displayed natural resistance characteristics with higher-yielding but more susceptible varieties, can take several years to produce new, stable varieties. This includes a myriad of crossing cycles, selections, and field testing under many different environmental conditions to check on the stability of the new varieties' resistance traits and agronomic viability. Genetic complexity resulting from sugarcane being a highly polyploid crop makes it difficult to breed. Polyploidy refers to having more than one gene copy; thus, it is hard to analyze the genetic composition and select genes related to resistance. This can be improved with MAS and genomic selection

techniques, where such a process may be speeded up by identifying genes responsible for resistance faster and more accurately than other conventional techniques (Chao et al. 1990).

However, sophisticated breeding techniques require vast inputs in technology, human capital, and infrastructural investments in research. Further, after producing such a resistant variety, many field tests must be conducted to evaluate performance in reality and track any unknown aftermaths, as could happen with its impact on the yield and quality of the sugar content. Other challenges and delays may come when seeking regulatory approvals and obtaining acceptability from farmers and the sugar industry (Xu et al. 1994). Other limitations include technical and logistical constraints. Sugarcane pathogens can evolve. For example, the sugarcane smut fungus can become resistant, offering potential bypasses of resistance traits bred into the varieties. This type of crop breeding and pathogen evolution arms race calls for continuous surveillance of the pathogen populations and regular adjustments to breeding strategy. Developing new, resistant sugarcane varieties against smut is a dynamic and challenging process involving many disciplines, such as genetics, plant pathology, agronomy, and environmental science. It requires patience and precision and incorporates considerable resources. Still, potential benefits such as crop stability, yield, and profitability are enormous (Churchill et al. 2006), as well as the effect of *S. scitamineum* infection on normal sugarcane plants (Fig. 20.5).

20.11 Current Strategies in China

Sugarcane is essential in China because it provides a significant share of the sugar market, and other industries, such as biofuels and paper, depend on it (Li et al. 2024). However, the major threat to production is sugarcane smut disease, and this challenge is being seriously combated through strategic breeding programs and policy initiatives by the government and various research institutions.

20.11.1 Government and Institutional Efforts

The Chinese government acknowledges that sugarcane has a strategic position in agriculture and, therefore, has implemented several policies to enhance the disease resistance and breeding of sugarcane. This is coordinated by a mix of national agricultural policies, funding mechanisms, and research programs for developing smut-resistant sugarcane varieties as well as improving general crop management (Byther and Steiner 1974). Research and Development: This government's strategy has supported extensive research and development activities. High involvement is also recorded from institutions such as the Chinese Academy of Agricultural Sciences and other agricultural universities in sugarcane genetics research across the country. The key emphasis is on identifying genetic markers associated with resistance traits,

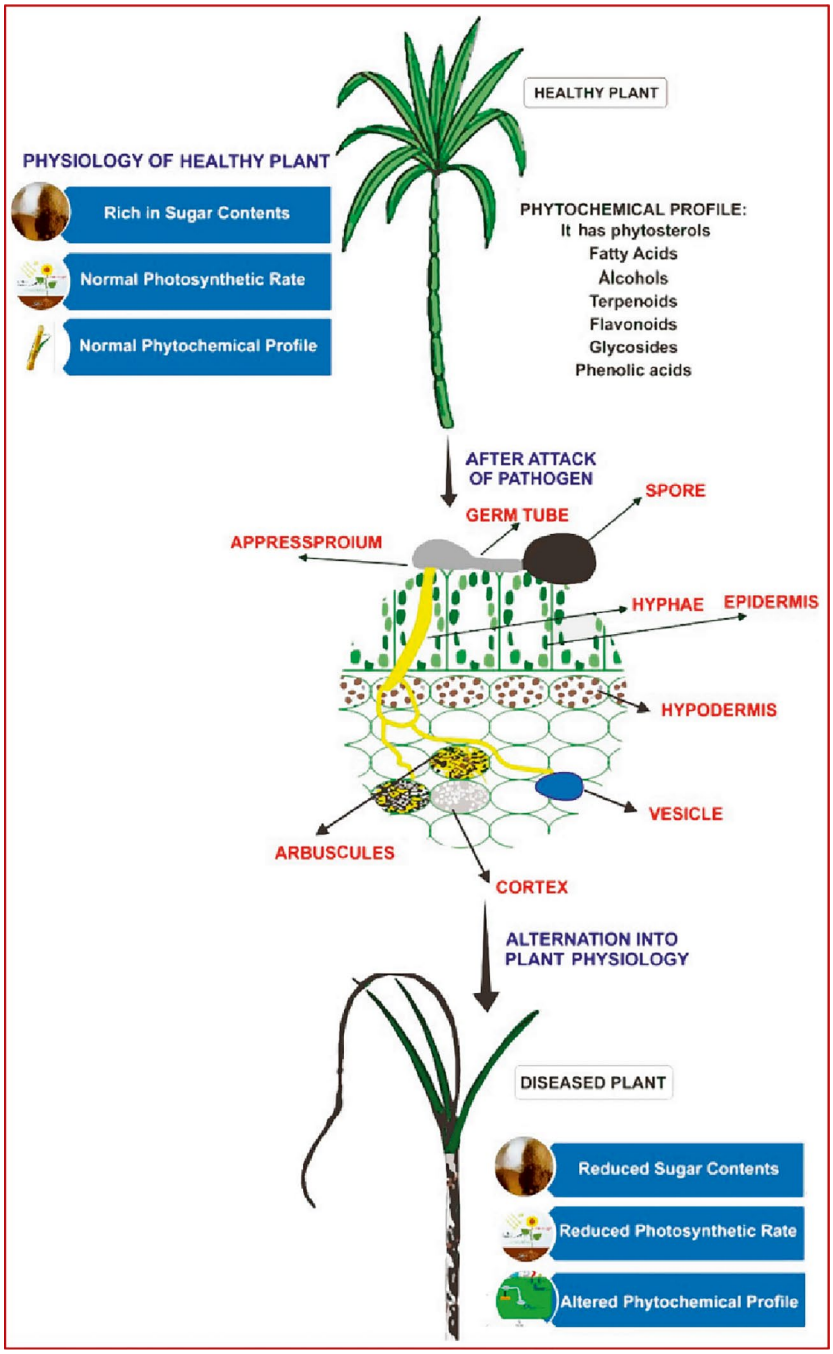

Fig. 20.5 Response of *Sporisorium scitamineum* infection on sugarcane plants (Rajput et al. 2021)

understanding the biology of the pathogen, and developing technologies that can rapidly breed varieties resistant to smut. Advanced techniques, such as genomic selection and gene editing are emphasized to speed up breeding (Srivastava et al. 2003).

Collaboration and Information Sharing: The study underlines a high level of cooperation between research institutions, government agencies, and private industry. Cooperation facilitates handling the information flows from the research labs to sugarcane farms. Regional agricultural extension services help in this regard, which enables new knowledge and technology to be passed on from the research labs to farmers with new resistant varieties and cultivation practices that help reduce risk due to the disease. Funding and Subsidies: The Chinese government funds and subsidizes sugarcane breeding programs, especially those aimed at developing disease-resistant varieties. Such funding and subsidies help to reduce the economic risk associated with research and breeding, mainly when focusing on long-term solutions such as resistance breeding, which may not yield immediate commercial returns. Policy support and regulations: Support policy through laws that facilitate safe, productive, and sustainable agriculture, which reduces the impacts of diseases, in this case, smut in sugarcane. Fungicides, among other chemical controls, are controlled stringently. More sustainable approaches, such as IPM, have become more pronounced. The government continues enforcing quarantine practices and crop sanitation that checks disease spread between regions (Singh et al. 2004).

20.11.2 Challenges to Sugarcane Breeding in China

Despite these concerted efforts, several challenges persist in breeding sugarcane varieties resistant to smut in China. Genetic Complexity of Sugarcane: The highly polyploid genome of sugarcane complicates the breeding process, as isolating and selecting desirable traits, such as disease resistance, proves challenging. This complexity necessitates sophisticated genetic tools and a deeper understanding of the sugarcane genome that is still under development (Bhuiyan et al. 2021).Pathogen Evolution: This evolving pathogen can quickly break out of resistance traits bred in new varieties. Continuous challenge by the pathogen necessitates regular monitoring of the pathogen population and updating the breeding strategy (Chassot et al. 2007).

20.11.3 Climatological and Environmental Factors

Climatological variations may affect sugarcane biology and alter the pattern of pathogens. Improving climatic conditions in the sense of higher temperatures and increased moisture could also facilitate the pathogen so that breeding and cultural

measures would need to be adjusted to accept the change. Farmer's adoption, even in the best cases with a resistant variety developed, is relatively slow. Most farmers find new things to be costlier and unknown or fear that new practices will change. Mass outreach and education on why certain varieties must be taken for planting could take a long time in terms of their implementation and usage by most of these farmers. There are a series of reasons why combating sugarcane smut disease has, despite attempts from the government and players in institutions, not lived up to real sustainable expectations. To keep up success, a series of repeated and adaptive approaches need to be upheld; among those are ongoing research collaboration fosterment and ensuring practicable solutions reach the most susceptible farmers (Chao et al. 1989).

20.11.4 Collaboration with International Research

International cooperation has dramatically enhanced China's work on the control of sugarcane smut disease. This partnership involves exchanging scientific information, conducting mutual research to improve both parties and sharing technologies. These collaborations work mutually to enhance resistance breeding and disease management techniques to enhance performance. International Research Partnerships—China has formed strategic partnerships with international agricultural research centers or universities that specialize in sugarcane research. Some of the prominent collaborations are with the International Sugar Organization and other research institutions in the agricultural fields of Brazil, India, and Australia, which are large sugarcane producers with a long history of disease management for sugarcane. These collaborations have mainly involved exchanging genetic resources, such as germplasm with resistance traits and more advanced biotechnological tools that may accelerate breeding for resistant varieties (Abera et al. 2009).

Technology Transfer and Shared Expertise: International collaborations provide Chinese scientists access to the latest technologies, such as genomic sequencing and CRISPR-based gene-editing tools, which are essential for identifying resistance genes and understanding the genetic makeup of sugarcane and its pathogens. In addition, training and capacity-building programs are integral to these collaborations, whereby Chinese scientists and researchers are trained on the latest methodologies in plant breeding and disease management (Gillaspie Jr et al. 1983). Joint Research Activities: Several joint research projects have been conducted concentrating on the genetic mechanisms conferring disease resistance and the development of effective integrated pest management tailored to various environmental situations. These activities usually entail publications of collaborative research discoveries and developing new sugarcane variety strains aimed explicitly at their environment's climatic condition or soil type in Chinese sugarcane fields.

20.11.5 Case Studies of Success and Ongoing Projects

Case Study 1: Development of the GXB9-10 Variety: It is an international collaborative achievement in developing the sugarcane variety GXB9-10. It was developed during a collaborative research project among Chinese agricultural researchers and their Brazilian counterparts (Scortecci et al. 2012). This variety showed excellent resistance against sugarcane smut under field tests carried out in southern China, an area where this disease particularly has high intensity. It also features high sugar content and yield and is thus preferred among farmers (Xing 2013).

Case Study 2: The China-Cuba Sugarcane Research Initiative: This is an ongoing research program undertaken by China and Cuba to enhance the genetic diversity of Chinese sugarcane varieties. This research focuses on the exchange of sugarcane germplasm that has shown resistance to various diseases, including smut. Cuban sugarcane varieties are very robust and resistant to disease, and this research program enables these characteristics to be introduced into Chinese varieties through cross-breeding and genetic evaluation (Gong et al. 1996).

Case Study 3: Joint Laboratory for Sugarcane Research: This joint laboratory collaborates with Chinese agricultural research institutes and the University of Queensland in Australia. They specialize in the molecular biology of sugarcane diseases, such as smut. The laboratory identified some key genetic markers related to disease resistance and has focused on developing transgenic sugarcane varieties to express these resistance traits more consistently (Abera et al. 2009).These case studies point out that, through international cooperation, the capability of sugarcane research in China has improved considerably. Further, they contribute to the global knowledge of disease management of sugarcane. Research and breeding programs continue to seek the boundaries further scientifically to breed and provide future generations with disease-resistant varieties that ensure solutions in the face of current and future problems.

20.12 Strategies for Enhancing Resistance in Future Breeding

Developing a strategy to improve resistance will be the cornerstone of further breeding programs to mitigate sugarcane smut, a significant sugarcane challenge in China. In that regard, advances in molecular breeding techniques are of particular importance. These allow precision and efficiency in the development of disease-resistant sugarcane varieties.

20.12.1 Advances in Molecular Breeding Techniques

Genomic Selection (GS): Genomic selection has revolutionized breeding complex traits such as resistance to disease in crops. For example, the breeding cycles are long, and the polyploid genetics of the plant are complex; therefore, GS could accelerate the development of smut-resistant varieties in sugarcane. DNA markers in the entire genome enable very accurate prediction of breeding values for seedlings or clones even before maturity. This technique allows selection, at the DNA level, to directly pick genetic individuals for their potential to express resistance characteristics, thus saving enormous amounts of time and resources on field testing (Que et al. 2011). CRISPR/Cas Genome Editing—This technology can directly modulate or knock out such specific genes related to susceptibility to smut disease in sugarcane. The ability of CRISPR/Cas to introduce targeted mutations in the DNA of sugarcane cells has recently been under study by Chinese researchers to enhance innate immune responses or disrupt disease pathways. A general description of the genome editing in sugarcane plants to produce elite varieties against biotic and abiotic stress tolerance (Fig. 20.6). This approach is far removed from the traditional genetic modification methods. It allows for high precision with minimal off-target effects, as edits are done without introducing foreign DNA into the plant (Karen et al. 2013).

20.12.1.1 Marker-Assisted Selection

This is the other high-power molecular breeding strategy applied for efficiently selecting plants with a particular trait of interest. Using MAS, breeders can rapidly screen large sugarcane populations for resistance to smut using specific DNA

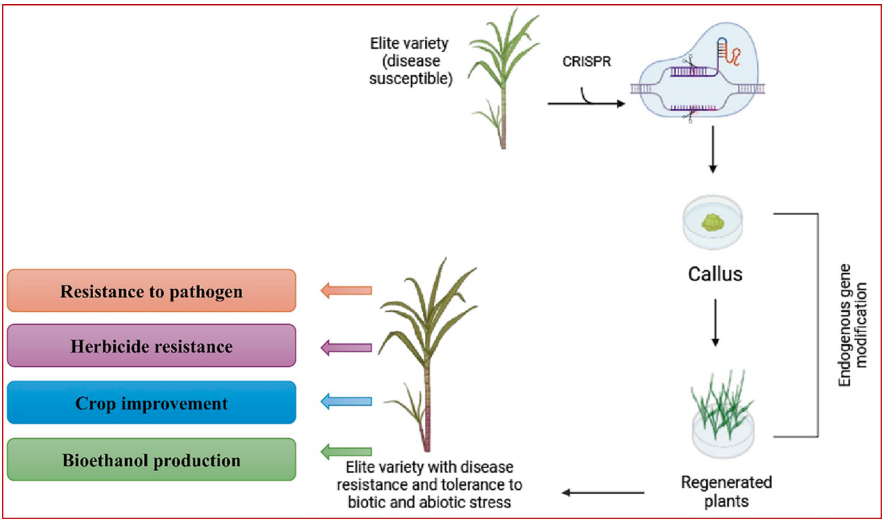

Fig. 20.6 A general description of the genome editing in sugarcane plants to produce elite varieties against biotic and abiotic stress tolerance

markers linked to genes conferring resistance. It is beneficial in the context of a backcrossing program in which the primary objective is the transfer of resistance traits into high-yielding commercial varieties from wild or non-commercial sources. It minimizes losses in the important agronomic traits because it ensures the transfer of only those genes associated with the resistance (Schenck 1998).

20.12.1.2 High-Throughput Phenotyping and Genotyping

Advances in recent years in phenotyping and genotyping technologies have permitted breeders to gather tremendous amounts of data on sugarcane plants' genetic makeup and physical nature. High-throughput systems, which involve automated imaging and analysis, are utilized to screen for disease symptoms in thousands of plants; thus, the data yield is very accurate and may be associated with genomic information. Integrating the two data sets enhances the selection precision in breeding programs (Zhang et al. 2015).

20.13 Traditional and Modern Approaches to Breeding for Smut Resistance

Traditional breeding, involving hybridization and selection, makes up the core of Chinese smut-resistant sugarcane breeding. This typically involves crossing susceptible and resistant varieties to combine desirable characteristics, followed by extensive field testing to select enhanced resistant offspring. While traditional approaches can be effective, they are often cumbersome and may require numerous iterations to achieve stabilization. Nevertheless, these approaches have helped develop commercial sugarcane varieties with moderate smut resistance. At the same time, novel techniques such as molecular breeding and genomic selection are being used to revolutionize smut resistance improvement. The marker-assisted selection allows breeders to identify specific genetic markers for resistance traits, enabling a rapid screening of large breeding populations. Genome-wide association studies are also possible, even achievable, with NGS technologies, thus providing the precise identification of resistance genes. The power of CRISPR-Cas9 gene editing has even been used in a novel approach to directly modify the genes related to resistance against smut, thereby speeding up the creation of resistant varieties. With such advanced techniques coupled with the traditional breeding method, it is within the Chinese researchers' power to create sugarcane varieties that are more robust and resistant (Dalvi et al. 2012).

20.13.1 Predicting and Preparing for Future Threats

As the pathogen evolves, *S. scitamineum*, new virulent strains of sugarcane smut will appear that might challenge resistance breeding continuously. Predictive tools, including disease modeling and surveillance systems, must be utilized to outsmart this possibility. Built from historical data of outbreaks of smut, disease forecasting models identify the region at a higher risk of future epidemics based on environmental factors. In addition, through molecular surveillance of pathogen populations, changes in virulence can be monitored, and responses can be initiated in breeding programs. Preparing for such threats in the future also requires proactive management of genetic diversity in breeding. Maintaining and expanding the genetic base of sugarcane collections will provide the breeder with a broader base of resistance genes. Further strength in the crop may be obtained by introducing novel resistance genes from wild sugarcane relatives and other compatible species through gene introgression. International collaboration with sugarcane research institutes can help Chinese researchers gain valuable insight into emerging disease trends as well as access global resources of germplasm to stay ahead of the fight against smut (Li et al. 2012).

20.13.2 Strategic Roadmap for Smut Resistance in China

An integrated, long-term resistance strategy against sugarcane smut is impossible without a multidisciplinary approach. For China, this plan should begin with an upgrade in investment for both conventional and innovative breeding infrastructure. Funding for molecular breeding programs is necessary, as well as enhancing and developing germplasm collections and sophisticated disease monitoring systems. Next, better coordination must be arranged among research institutions, agencies, and sugarcane growers. National and regional breeding centers handling varieties resistant to specific diseases would facilitate the process. These centers, too, have to constantly interact with growers at the subregional level to assist them in producing resistant varieties and guidance on best-practice technologies for disease control, getting new technologies directly to farmers' fields (Churchill et al. 2006). Long-term sustainability also requires embedding environmental and ecological considerations into the breeding strategy. Sugarcane varieties are both resistant to diseases and drought tolerant, with increased nutrient-use efficiency, ensuring performance well in conditions experienced to prevail in many parts of China. Such an all-round breeding approach will make sugarcane crops more resilient, shielding the industry from future threats.

20.14 Future Prospective

One of the significant hurdles in the sugarcane cultivation business of China over the past years has been the presence of the smut disease caused by *S. scitamineum.* It caused stunted growth, less yield, and lower plant sugar content. The danger of its loss would imperil the industry. Smut has recently become the focus point for research in breeding for resistance in sugarcane. More resistance is made to traditional and new methods. The prediction of future threats and preparedness must continue. Strategic direction is needed for the long-term maintenance of the sugarcane production system in China and for decreasing the risk created by the disease of smut. A lot of contributions in resistant varieties of sugarcane have been made through the use of traditional breeding approaches. Methods include a selection of the mother plant with desirable characteristics from which offspring is developed from the cross-bred flowers. The whole process is a long and time-consuming procedure that usually takes years, as each generation has to be evaluated based on disease resistance and agronomic traits, such as yield and sugar content (Lloyd and Pillay 1980).

Mass selection, recurrent selection, and progeny testing are the methods employed in China to improve the resistance against smut disease. Some progress has been possible with traditional approaches, though these are relatively slow processes and less effective with the faster-evolving pathogens (Waller 1970). The recent biotechnological approaches are becoming increasingly prominent in China to surpass the limitations of traditional breeding. MAS has reduced breeding cycles by enabling breeders to detect resistance genes during the initial breeding stages. Genomic selection and gene editing technologies, such as CRISPR-Cas9, are being pursued to introduce resistance to smut more efficiently and precisely. These tools can identify the specific genetic markers associated with resistance to S. Biotechnology can also be used to select improvements in the genome of sugarcane specifically; it allows the transfer of resistance genes from wild relatives of sugarcane into the crop and increases genetic diversity for breeding programs (Yao et al. 2005).

The advance of smut pathogens requires prediction and preparedness for new threats. Monitoring the genetic diversity of *S. scitamineum* populations in the sugarcane fields in China may help detect the emergence of new virulent strains before their spread. These surveillance systems, coupled with predictive models, can help breeders predict virulence changes of pathogens and adjust their breeding accordingly. In addition to that, a better molecular-level interaction between sugarcane and its pathogen might provide new mechanisms that could be used in later varieties. A strategic roadmap is needed for the long-term sustainability of smut resistance breeding in China (Sundar et al. 2012). This will integrate traditional and modern approaches, thus capitalizing on the strengths of each approach. First, conventional breeding programs will be continued and supplemented with advanced genomic tools to hasten the rate of resistance development.

The parallel effort will foster collaboration between research institutions, universities, and sugarcane growers. This will be crucial for sharing knowledge, resources, and genetic material. A robust pathogen monitoring system is essential. Regular data collection on the populations of smut pathogens will enable researchers to be one step ahead of the outbreak and adjust their breeding programs to meet new challenges. Predictive modelling would complement this system, allowing breeders to focus on long-term sustainability rather than simply reacting to crises as they arise (Song et al. 2012).Thirdly, there is a need to broaden the germplasm of sugarcane by exploiting its less explored wild relatives and landraces. Diversity in the genetic base of sugarcane varieties increases resistance against smut and emerging diseases or stresses. These diverse genes can be integrated into the breeding pipeline, and then the future sugarcane varieties will be strong enough against evolving pathogens. Finally, innovation and international cooperation should be promoted. The country can learn how to share expertise and research with other countries where sugarcane is grown, most of which share the same problems with the disease. Such cooperation could expedite the discovery of novel resistance genes and the efficiency of sugarcane breeding programs worldwide. This increased resistance towards sugarcane smut disease demands a diversified strategy that involves traditional breeding alongside new technologies involving biotechnology, disease surveillance, and strategic collaborations. China should predict threats, prepare to counter these later, and ensure the safety of its sugarcane sector from diseases such as smut and secure successful production over the future decades (Singh et al. 2014).

20.15 Conclusion

The struggle against sugarcane smut disease in China underscores the need for continuous research and development to maintain the productivity and security of the agricultural industry. Sugarcane is one of the top crops for domestic consumption and industrial use, and this smut disease directly threatens its yield and quality. Without sustained efforts toward developing resistance varieties, epidemics are likely to affect the industry significantly, and significant losses are expected both in economic and food security terms. Thus, while maintaining the short-term protection of the crop, continued R&D efforts have also been a vital stone pillar for the long term to ensure the sustainability of the agriculture sector. This investment in research has successfully paved the way for a greater understanding of the nature of the pathogen *S. scitamineum* and its mechanism as it evolves along with interacting sugarcane varieties. Such knowledge will form the bedrock of breeding programs, ensuring that they remain ahead of the newly emerging strains of smut. No less significant are advances concerning the biotechnological tools of gene editing and marker-assisted selection in that these accelerate breeding with great precision. This involves selecting and introducing desirable genes that may otherwise only manifest after many years and decades through normal traditional routes.

Continued research also provides more opportunities to study the wild relatives of sugarcane and underutilized genetic resources, thus expanding the pool of resistance traits for future breeding. Overall, continued R&D ensures breeders have the tools, knowledge, and resources available to manage current and future disease pressures. Another is that sugarcane breeding will necessitate increased involvement by international collaborations. In regions of the world, challenges arising from smut and other diseases have been increasingly similar. Germplasm and information from different breeds can then be tapped into once these breeders pool together to access one another. International networks that focus research efforts on this crop can, therefore, accelerate their breakthrough in developing resistance genes and desirable traits. Such collaborations will be helpful for the countries where sugarcane constitutes their prime crop, at least for the Chinese, who could avail themselves of a much bigger pool of expertise and a more significant number of genetic resources. Thus, the race to battle Chinese sugarcane smut is way too advanced, but ongoing R&D with an integrated approach toward breeding and international collaborative approaches assures durable resistance at higher chances. The sugarcane of China's tomorrow depends on continued investments in R&D; if properly strategized, it could also manage this massive sector of China on proper grounds against smut attacks.

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Chapter 21
Sugarcane White Leaf Disease Resistance: A Challenge to Sugarcane Breeding in India

M. Nivedha, M. Elangovan, T. Praveen, and Nguyen Bao Quoc

Abstract Sugarcane white leaf disease (SCWLD), caused by sugarcane white leaf phytoplasma, poses a critical threat to sugarcane production in Southeast Asia, leading to substantial yield losses. SCWLD, first reported in Thailand in 1954, manifests as cream-colored leaf streaks and stunted growth, primarily transmitted by the leafhopper *Matsumuratettix hiroglyphicus*. Disease management relies on developing resistant cultivars through breeding, yet the sugarcane complex polyploid genome, mixed infections, and variability in pathogen and vector strains present challenges. Wild relatives, including *Saccharum spontaneum* and *Erianthus* spp., offer potential resistance traits supported by germplasm collections. Integrating resistance breeding with IPM strategies and healthy planting materials is essential for effective management. This book chapter delves into the detailed study of developing resistant sugarcane cultivars, exploring current research efforts, obstacles faced in breeding programs, and potential strategies for overcoming these challenges to ensure sustainable sugarcane production in India.

Keywords Sugarcane white leaf disease · Phytoplasma · Resistance breeding · Integrated pest management

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21.1 Introduction

Sugarcane white leaf disease (SCWLD), caused by the *sugarcane white leaf phytoplasma* (16SrXI group), significantly impacts sugarcane production in Southeast Asia. This disease is one of the most severe threats to sugarcane crops, leading to drastic yield reductions and poor sugar quality. SCWLD was first reported in Thailand in 1954 (Marcone 2002) and has been widely distributed in sugarcane-producing countries, such as India, Pakistan, Sri Lanka, The Philippines, Myanmar, and Vietnam (Kumarasinghe and Jones 2001; Wang et al. 2015). Globally, the implications of SCWLD extend beyond localized economic losses. The disease threatens food security and livelihood for millions of people involved in sugarcane cultivation. In India, SCWLD is primarily observed in Uttar Pradesh, Maharashtra, Tamil Nadu, and Karnataka, significantly affecting sugarcane production.

The symptoms of SCWLD can lead to severe reductions in crop yield and quality, posing economic risks to farmers and the sugar industry. As the demand for sugar rises, the urgency to develop resistant sugarcane varieties becomes paramount. However, breeding for resistance to SCWLD presents unique challenges due to the complex nature of the disease and its interactions with host plants and insect vectors. This chapter delves into the detailed study of developing resistant sugarcane cultivars, exploring current research efforts, obstacles faced in breeding programs, and potential strategies for overcoming these challenges to ensure sustainable sugarcane production in India.

21.2 Sugarcane White Leaf Disease

The initial symptoms of sugarcane white leaf disease (SCWLD) are characterized by the appearance of cream-colored or white stripes that run parallel to the midribs of the leaves. As the disease progresses, these stripes may coalesce, extending along the entire leaf blade and leading to severe chlorosis (yellowing) of the leaves. Affected leaves are typically narrower and more petite than healthy plants, exhibiting a soft texture. The shoots become slender and chlorotic, often tufted at the tips of slowly growing shoots (Song et al. 2021). Additionally, there is excessive development of proliferating tillers with shortened internodes, resulting in a bushy, broom-like appearance as the plant attempts to compensate for the loss of vigor caused by the disease.

As the pathogen advances, more severe symptoms manifest, including stunted growth and the absence of lateral shoots on the upper parts of infected stalks. Severely diseased plants often fail to set fruit, leading to eventual plant death. The total chlorophyll content in affected plants can decrease by up to 90%, drastically affecting photosynthesis and overall plant health (Song et al. 2019; Verma et al. 2024a). Other observations include dieback at the growing points and distortion of inflorescences, contributing to a general decline in plant vigor that typically results

in death shortly after symptoms appear (Leetanasaksakul et al. 2022; Narmilan et al. 2022; Verma et al. 2024b).

Detection of LWD in sugarcane can be effectively conducted through both field and laboratory methods. Field detection relies on visual inspection of symptoms, such as leaf yellowing, stunted growth, and shortened internodes, along with monitoring leafhopper populations, the known disease vectors. Advanced techniques like remote sensing using UAVs equipped with multispectral imagery can analyze plant health and identify infected areas. In laboratory settings, even at low concentrations, molecular methods, such as RT-qPCR (Reverse Transcription Quantitative Polymerase Chain Reaction), detect and quantify the phytoplasma associated with SCLWD. Additionally, loop-mediated isothermal amplification (LAMP) has emerged as a rapid diagnostic tool suitable for field applications, capable of detecting phytoplasmas within an hour. Proteomic approaches, including quantitative label-free nano-liquid chromatography-tandem mass spectrometry (nano LC-MS/MS), help identify potential protein biomarkers associated with SCLWD susceptibility, enhancing our understanding of plant defense mechanisms and facilitating the development of resistant sugarcane varieties (Leetanasaksakul et al. 2022).

21.2.1 Transmission of SCWLD

The vectors responsible for transmitting SCWL include the leafhoppers *Matsumuratettix hiroglyphicus* and *Yamatotettix flavovittatus*, which facilitate the spread of the disease within infected crops and healthy plants. The most important primary vector for the transmission is *Matsumuratettix hiroglyphicus*. When these leafhoppers feed on infected sugarcane plants, they acquire the phytoplasma through piercing mouthparts. This process involves an acquisition access period (AAP) during which the insect must feed on the infected plant to develop the pathogen. After a latent period, the phytoplasma is transmitted to healthy plants during subsequent feedings. Research has demonstrated that *M. hiroglyphicus* transmits the disease transovarially, allowing the pathogen to persist within leafhopper populations across generations. This characteristic makes controlling SCWLD particularly challenging, as the vectors can carry and spread the disease without new hosts (Hanboonsong et al. 2002).

In addition to *M. hiroglyphicus*, other leafhopper species, such as *Yamatotettix flavovittatus*, have also been identified as vectors of SCWLD, with varying transmission efficiencies. Studies have shown that *M. hiroglyphicus* has a higher transmission efficiency (55%) than *Y. flavovittatus* (45%). Multiple vectors complicate management efforts, as these species peak at different times of the year and can complement each other in spreading the disease (Hanboonsong et al. 2006).

21.2.2 Sugarcane Resistance Mechanism Against SCWLD

Resistance mechanisms in sugarcane against SCWLD involve both biochemical and molecular responses. Recent studies have identified that sugarcane plants exhibit systemic acquired resistance (SAR) through the application of salicylic acid (SA) and benzothiadiazole (BTH). These treatments have been shown to increase total phenolic compounds and reduce phytoplasma concentrations in infected plants, with BTH at a concentration of 2.4 mM demonstrating particularly effective results in lowering phytoplasma levels compared to untreated controls (Ratchaseema et al. 2021). Additionally, proteomic analyses have highlighted specific proteins associated with reduced susceptibility to SCWLD in certain sugarcane varieties. For instance, two candidate protein biomarkers were identified in the resistant variety UT1, linked to functions such as protein folding and metal ion binding, which may play crucial roles in enhancing the plant defense mechanisms against phytoplasma infection (Leetanasaksakul et al. 2022).

Moreover, transcriptomic profiling of sugarcane during SCWLD infection has revealed significant alterations in gene expression related to various metabolic pathways, including those involved in secondary metabolite biosynthesis and plant hormone signaling transduction. The upregulation of genes associated with phytoalexin production and reactive oxygen species (ROS) accumulation suggests a complex defense response activated by the pathogen (Guo et al. 2021; Lohmaneeratana et al. 2024). The interplay between enhanced lignin biosynthesis and the expression of specific defense-related genes further reinforces the plant's ability to resist pathogen invasion (Singh et al. 2020).

Genetic resistance in sugarcane varieties against SCWLD has been a significant research focus. Studies have identified quantitative trait loci (QTLs) associated with resistance, such as those linked to sugarcane mosaic virus resistance, which provide valuable insights for breeding programs (Lu et al. 2023). Specific genes, including ScDIR, have been shown to enhance lignin biosynthesis and improve resistance to pathogens, such as *Sporisorium scitamineum*, with transgenic overexpression resulting in increased lignin content and disease resistance (Li et al. 2024a). Additionally, integrating molecular markers linked to disease resistance traits into breeding programs facilitates more efficient selection processes and accelerates the development of resilient sugarcane varieties.

21.3 Sugarcane Breeding in India

Sugarcane breeding in India has evolved significantly over the years, particularly in response to biotic and abiotic stresses. The focus on disease resistance is paramount, as sugarcane is susceptible to various pathogens, including viral diseases, such as sugarcane mosaic virus (SCMV) and sugarcane streak mosaic virus (SCSMV), as well as fungal infections, such as smut caused by *Sporisorium scitamineum*. The

traditional breeding programs have utilized both hybridization and selection methods to develop varieties that exhibit resistance to these diseases. For instance, a recent study screened 129 sugarcane genotypes for resistance to SCMV, SCSMV, and sugarcane bacilliform virus (SCBV), revealing that only eight genotypes were free from mosaic viruses. This highlights the widespread prevalence of these diseases in Indian sugarcane crops, necessitating the identification and incorporation of resistant germplasm into breeding programs to enhance crop resilience (Vamsi Krishna et al. 2023).

In addition to disease resistance, sugarcane breeding efforts address abiotic stresses, such as drought, salinity, and extreme temperatures. Incorporating genes from wild relatives and using advanced genomic techniques have proven effective in developing stress-tolerant varieties (Afghan et al. 2024; Liang et al. 2024). For example, introgression breeding has been employed to enhance drought tolerance in sugarcane by utilizing genetic resources from related species (Wu et al. 2022). Genomic approaches, including transcriptomics and identifying novel microRNAs, have further elucidated the genetic pathways involved in abiotic stress responses (Xu et al. 2022). For example, introducing *Saccharum spontaneum* into breeding programs has provided valuable traits that improve biotic and abiotic stress resistance (Li et al. 2023). Research has shown that sugarcane cultivars with higher levels of certain phytohormones, such as jasmonic acid and salicylic acid, exhibit better resistance to smut disease. Furthermore, endophytic microbial communities in non-susceptible cultivars have been found to enhance disease resistance through antagonistic interactions with pathogens (Guo et al. 2023). A notable success story in Indian sugarcane breeding is the development of the Co 86032 variety, which has shown significant resistance to multiple diseases while maintaining high yield potential. This variety is a benchmark for future breeding efforts to combine disease resistance with agronomic performance.

Breeding for resistance to both biotic and abiotic stresses in sugarcane enhances crop resilience and productivity (Li et al. 2024a, b). Biotic stresses, including diseases and pests, significantly impact sugarcane yields (Li et al. 2023). Over 100 pathogens threaten sugarcane cultivation, such as *Sporisorium scitamineum*, *Xanthomonas albilineans*, and various nematodes. Research has identified various resistance mechanisms, including external barriers (such as lignin accumulation) and internal biochemical responses (such as activating defense-related genes). For instance, studies have shown that transgenic sugarcane expressing ScDIR genes, which are involved in lignin biosynthesis, exhibit enhanced resistance to smut disease by reinforcing cell wall defense (Li et al. 2024a, b). Identifying genes associated with disease resistance can facilitate marker-assisted selection in breeding programs, allowing for the development of cultivars that combine multiple resistance traits to combat various pathogens effectively (Verma et al. 2022).

These strategies improve sugarcane resilience to environmental stresses and enhance its overall agronomic performance. As climate change continues to pose challenges to agriculture (Verma et al. 2024c), integrating biotic and abiotic resistance traits into sugarcane breeding programs will be crucial for sustainable production (Song et al. 2022; Huang et al. 2024).

21.4 Potential Sources of Resistance from Wild Relatives and Germplasm

The search for effective resistance to white leaf disease (WLD) in sugarcane cultivars has led researchers to explore wild relatives and diverse germplasm collections as potential sources of resistance traits. Sugarcane, particularly *Saccharum spontaneum* and *Erianthus* spp., are important wild relatives exhibiting natural resistance to various biotic and abiotic stresses, including WLD. *S. spontaneum* is known for its adaptability and resilience, thriving under adverse environmental conditions, while *Erianthus* spp. displays high biomass production and multiple pest resistances. These species are essential for breeding programs aimed at enhancing both productivity and disease resistance in cultivated sugarcane varieties (Park et al. 2024).

Germplasm collections, such as the World Collection of Sugarcane and Related Grasses (WCSRG), play a critical role in identifying and harnessing genetic diversity for resistance breeding. The WCSRG includes a wide array of *Saccharum* species and related genera, such as *Miscanthus* and *Tripidium*, which provide genetic resources that can be introduced into commercial sugarcane varieties. Recent studies employing molecular markers have pinpointed genotypes within these collections that harbor resistance-associated genes, which can be leveraged to develop cultivars with enhanced resilience to SCWLD (Nair 2012). However, integrating wild relatives into breeding programs is not without challenges. The complex polyploidy of sugarcane complicates the transfer of traits and necessitates careful characterization of germplasm to maintain genetic integrity. Advances in molecular biology, such as genome-wide association studies (GWAS), are aiding in identifying beneficial alleles linked to disease resistance. These molecular tools are essential in facilitating the targeted breeding of sugarcane varieties that are high-yielding and resilient against diseases like SCWLD.

21.5 Challenges in Breeding Resistant Cultivars

Breeding sugarcane cultivars resistant to white leaf disease (WLD) is a complex challenge primarily due to the intricate genetic structure of sugarcane and the pathogenic nature of the disease caused by the sugarcane white leaf virus (SCWLV). This virus is transmitted by leafhoppers, complicating efforts to control its spread in the field. Additionally, WLD often occurs in mixed infections with other viruses, such as the sugarcane mosaic virus (SCMV) and sugarcane streak mosaic virus (SCSMV), making it difficult to isolate specific resistance traits for WLD. The impact of SCWLV significantly reduces crop yield and sugar quality, necessitating the identification of protein biomarkers that may enhance resistance. Recent studies have utilized proteomic analyses to compare the defense mechanisms of various sugarcane varieties against WLD. For instance, researchers identified two candidate

protein biomarkers associated with reduced susceptibility in a less affected variety, UT1, which could inform both molecular and conventional breeding strategies to improve resistance (Leetanasaksakul et al. 2022).

Geographical variability in disease expression adds another layer of complexity to breeding efforts. Varieties demonstrating resistance in one region may fail in another due to differing environmental conditions and pathogen populations. For example, the variety Co 205 has exhibited inconsistent resistance across locations in India, highlighting the necessity for localized breeding strategies. Furthermore, traditional breeding methods can be time-consuming, requiring multiple generations to evaluate resistance traits adequately. Modern approaches, such as marker-assisted selection (MAS) and genomic selection are being integrated into breeding programs to address these challenges. These techniques enable breeders to identify and select desirable traits more efficiently. Recent findings have identified elite sugarcane accessions with potential resistance to mosaic viruses, providing valuable resources for future breeding efforts focused on developing resilient cultivars against WLD and other viral threats.

21.5.1 Management of SCWLD

Managing the incidence of SCWLD is critical for maintaining sugarcane yields in India. The most effective strategy for managing this viral disease is developing and cultivating resistant sugarcane varieties. Recent research has highlighted the importance of identifying and utilizing resistant germplasm from cultivated varieties and wild relatives. A study screened 129 sugarcane genotypes for resistance to SCMV, SCSMV, and SCBV, revealing that only eight genotypes were free from these viruses, indicating a significant prevalence of viral infections in the field (Vamsi Krishna et al. 2023). This underscores the necessity for breeding programs to focus on incorporating these resistant sources into new varieties to enhance overall disease management.

In addition to breeding resistant varieties, cultural practices play a vital role in controlling SCWLD. Implementing healthy seed nursery programs that utilize virus-free planting material is crucial, as this can significantly reduce the incidence of SCWLV in the field. Furthermore, cultural practices, such as crop rotation and field sanitation can help minimize the spread of the virus by disrupting the life cycle of leafhoppers, the primary vectors of SCWLV. Integrated pest management strategies that include monitoring pest populations and applying insecticides judiciously are also essential for managing leafhopper populations effectively. These combined efforts aim to reduce the impact of SCWLD and contribute to sustainable sugarcane production in India.

Hot water and tetracycline treatments have been explored as methods to manage SCWLD. Research indicates that treating sugarcane setts with hot water alone cannot eliminate the SCWL phytoplasma. For instance, studies have shown that dipping infected cane setts in hot water at 50 °C for 2–4 hours or 53 °C for 1 hour does

not effectively eradicate the pathogen. Some positive effects were observed when setts were treated at 52 °C for 8 hours or 54 °C for 40 minutes, but most setts failed to produce regular shoot buds due to the stress of such high temperatures. In contrast, tetracycline treatments have yielded better results; treatment with tetracycline for 72 hours significantly reduced phytoplasma multiplication, allowing the growing shoots to remain symptom-free for up to 3 months.

Moreover, disease-free plant production through tissue culture techniques has proven effective in controlling SCWLD. Intensive programs in Taiwan utilizing conventional selection and tissue culture have successfully broken disease epidemics associated with SCWL phytoplasma. For example, meristem tip tissue culture has been used extensively in Thailand to produce disease-free plants. Additionally, sanitation practices, such as removing infected plants and debris, crop rotation, and disease-free cane setts, have been implemented to minimize the risk of reinfection. These strategies have contributed to controlling SWLD effectively in regions like Taiwan, where such integrated approaches have led to a significant reduction in disease incidence over time (Kaewmanee and Hanboonsong 2011).

One notable success story in managing white leaf disease in India is developing and promoting resistant sugarcane varieties. The variety Co 86032 has shown significant resistance to multiple viral diseases, including SCWLD. Its adoption among farmers has improved yields and reduced losses due to viral infections. This success highlights the effectiveness of breeding programs focusing on enhancing disease resistance through traditional breeding techniques combined with modern biotechnological approaches.

Chemical control methods against SCWLD face significant limitations that hinder their effectiveness and sustainability. One major challenge is the non-target impact of broad-spectrum insecticides, which can harm beneficial insects and disrupt natural pest control, potentially leading to pest resurgence and increased reliance on chemicals. Additionally, the development of resistance among pest populations due to the overuse of these chemicals diminishes their long-term efficacy (Ratchaseema et al. 2021). The timing and method of application further complicate chemical control; if insecticides are applied too late or too early, they may fail to manage leafhopper populations that transmit the virus effectively. Frequent reapplications are necessary, increasing costs and labor while posing environmental and health risks to farmworkers (Vamsi Krishna et al. 2023). Consequently, while chemical methods can provide immediate relief from pest pressures, they are not a sustainable long-term solution for managing SCWLD. Integrated pest management (IPM) strategies that combine biological controls, cultural practices, and resistant varieties are recommended as a more holistic approach to effectively manage pest populations while minimizing the negative impacts associated with chemical use (Verma et al. 2022).

Research on SCWLD has made significant strides, yet several gaps in the current knowledge hinder effective management and control strategies. One notable gap is the knowledge gap regarding the long-term effectiveness of various treatment methods, such as hot water and tetracycline applications. While studies have shown that soaking cane stalks in hot water followed by tetracycline can reduce phytoplasma

levels, there is limited understanding of how these treatments affect cane germination rates over extended periods. For example, while hot water treatments alone have reduced phytoplasma concentration, they also significantly lower germination rates, leading to questions about the balance between effective disease control and plant viability over time (Kaewmanee and Hanboonsong 2011). Despite extensive screening of numerous sugarcane varieties for resistance to SCWLD, no resistant cultivars have been identified. This absence of resistant varieties highlights a need for further research into the genetic and physiological mechanisms underlying susceptibility to SCWLD. Furthermore, while some studies have explored the use of disease-free plant propagation techniques, there is insufficient data on the long-term sustainability of these practices in various agro-climatic contexts (Verma et al. 2021, 2023a, b). Understanding how different cultivation practices, such as crop rotation and sanitation measures, interact with environmental factors to influence disease dynamics remains an underexplored area that could provide insights for better management strategies.

21.6 Future Prospects

Future research on SCWLD should focus on two critical areas: genetic studies and vector management strategies. Genetic research is essential for understanding the mechanisms of resistance or tolerance to SCWLD within sugarcane varieties, as extensive screening has yet to identify resistant cultivars. Advanced genomic techniques, such as genome-wide association studies (GWAS), could help identify potential resistance genes and inform breeding programs incorporating these traits into commercial varieties. Additionally, exploring the genetic resources of wild relatives, such as *Saccharum spontaneum*, may provide valuable insights for enhancing resistance. Concurrently, effective vector management strategies are crucial for controlling the spread of SCWLD. Integrated vector management (IVM) approaches should be developed to optimize resource use and enhance the efficacy of vector control measures, focusing on the ecology and behavior of leafhopper vectors responsible for transmitting the disease. Research should investigate targeted control methods, including biological control agents and environmental management practices while evaluating their effectiveness across agro-climatic conditions. By addressing these areas, future research can significantly mitigate the impact of SCWLD on sugarcane production.

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Chapter 22
Breeding Approaches and Genetic Engineering for Enhancing Sugarcane Tolerance to Borer Infestation

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Abstract Sugarcane (*Saccharum officinarum* L.) is a vital crop worldwide that encounters various challenges from pests, especially attacked by borer insects, which greatly affect the yield and quality of sugarcane. Stem borers are among the most destructive pests, which cause severe damage by tunnelling through plant tissues, weakening the crop, and providing entry points for pathogens. Developing resistant sugarcane varieties is a sustainable approach to managing borer pests, and breeding strategies are crucial in enhancing borer resistance. This chapter discusses the natural resistance mechanisms in sugarcane, emphasizing its physical and chemical defenses and behavioral resistance strategies. It also discusses screening techniques for identifying borer resistance, including bioassays and near-infrared reflectance spectroscopy (NIRS). Breeding approaches, from traditional methods to modern technologies, such as marker-assisted selection (MAS), genetic modification, and CRISPR/Cas9, are explored in detail. The role of wild relatives of sugarcane, which possess valuable genetic traits for pest resistance, is also highlighted as

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a key resource for improving resistance. This chapter concludes by emphasizing the need for integrated, multifaceted approaches in developing sugarcane varieties with enhanced borer resistance to reduce dependency on chemical control and promote sustainable farming practices.

Keywords Sugarcane · Tolerance · Borer · Breeding strategies

22.1 Introduction

Sugarcane (*Saccharum officinarum* L.) is a key crop in tropical and subtropical regions, serving as a primary raw material source for the global sugar and biofuel industries (Verma et al. 2024a, b). However, its cultivation is increasingly threatened by various pests, mainly borer insects. Sugarcane is subject to a range of biotic stressors, each of which can significantly impact its growth and yield (Srikanth et al. 2012; Dhaliwal et al. 2015; Carbognin et al. 2023; Geetha et al. 2022; Li et al. 2023; Ambethgar et al. 2024). Among these, lepidopteran borers are the most damaging pests in key sugarcane-producing regions, including Brazil, India, China, the USA, South Africa, Indonesia, Reunion Island, Mexico, Iran, Colombia, and Papua New Guinea. Around 50 species of Lepidoptera borers, from genera such as *Chilo, Diatraea, Sesamia, Argyroploce, Tryporyza, Scirpophaga, Eoreuma, Telchin*, and *Eldana,* have been identified as sugarcane pests. These species mainly belong to the Pyralidae, Crambidae, Noctuidae, and Tortricidae families (Ngcobo et al. 2023). The larvae of these borers feed on the plant's tissues, weakening its structural integrity, making it more vulnerable to diseases, and reducing the overall productivity.

To reduce the damaging impact of these pests, there is an urgent need for sustainable pest management strategies (Afghan et al. 2024). While chemical control methods, such as insecticide use, have been commonly adopted, they present significant environmental and economic challenges. These include harm to beneficial organisms, pest resistance development, and increased production costs (Khan et al. 2023). As a result, researchers and agriculturalists are increasingly turning to breeding innovations as a promising solution to enhance the natural defenses of sugarcane against borers (Malinowski et al. 2024). In this chapter, we will explore various breeding strategies to improve borer resistance in sugarcane. From traditional breeding techniques to cutting-edge biotechnological approaches (Li et al. 2023), the focus will be on how scientists and breeders enhance sugarcane plants' natural defenses to make them more resilient to these damaging pests. Enhancing resistance through breeding tools can help decrease reliance on chemical pesticides, boost crop yields, and promote more sustainable agricultural practices (Singh et al. 2019; Song et al. 2019).

22.2 Understanding Borer Pests in Sugarcane

Sugarcane is vulnerable to infestations by a wide range of insect species from orders, such as Lepidoptera, Homoptera, Coleoptera, Hemiptera, Orthoptera, and Isoptera worldwide. Lepidoptera borers are particularly significant pests in nearly all sugarcane-growing countries (Li et al. 2024a, b). A notable group of sugarcane borers belongs to the genus *Chilo*, which is classified under the Crambidae family, though it was previously categorized under Pyralidae (Li et al. 2024a, b). Species within the *Chilo* genus cause damage by creating "dead-heart" in sugarcane shoots and feeding on the internal stem tissues (Hajjar et al. 2023).

This feeding significantly reduces sugarcane yields and can even result in total crop failure (Ngcobo et al. 2023). One well-known species, *Chilo infuscatellus*, or the early shoot borer, causes damage to young sugarcane plants, leading to reduced yields (Casaroli et al. 2024). This species can survive year-round in mild climates (Casaroli et al. 2024). Another important species in this genus is the internode spotted borer, *Chilo sacchariphagus*, which typically attacks plants aged 3–7 months (Nikpay et al. 2020). It significantly impacts cane yield more than sucrose yield (Dutra et al. 2023). The third species, *Chilo venosatus*, is also a significant pest, contributing to substantial economic losses (Tanveer et al. 2024). *Chilo tumidicostalis* mainly damage sugarcane after internode formation (Li et al. 2024a, b).

The fifth species, *Chilo auricilius*, commonly known as the stalk borer, has young larvae that bore into sugarcane shoots and canes by creating holes and forming galleries within the stalk. This damage impairs plant growth, leading to losses in both cane yield and sugar recovery (Nikpay et al. 2023). In the *Diatraea* genus, borers primarily affect sugarcane in the Americas (Carbognin et al. 2023). One of the most notable species, *Diatraea saccharalis*, is widespread across the Western Hemisphere (Zafar 2024), with other species such as *D. albicrinella*, *D. busckella*, *D. tabernella*, *D. centrella*, *D. indigenella*, *D. lineolata*, *D. considerate*, and *D. magnifactella* also recorded as sugarcane pests (Zafar 2024). Pink borers within the *Sesamia* genus significantly reduce sugar recovery (Chandran et al. 2023). Among these, *Sesamia cretica* and *Sesamia nonagrioides* are widely distributed across sugarcane-growing regions, attacking the crop at all stages of growth (Horgan 2023), while *Sesamia grisescens* can cause considerable damage in certain countries (Subiyakto et al. 2023).

Sesamia inferens larvae bore into the aboveground parts of sugarcane seedlings, contributing to further damage (Mehdi et al. 2024). Additionally, *S. nonagrioides*, also known as the corn borer, is a significant pest within the *Sesamia* genus, severely affecting sugarcane yields (Ebrahimifar et al. 2023). In addition to the borers from *Chilo*, *Diatraea*, and *Sesamia*, other genera also contribute to sugarcane damage (Shang et al. 2024). For example, *Argyroploces chistaceana* larvae damage the plant's underground and surface parts, increasing population density and subsequent cane yield and sugar losses (Li et al. 2024a, b). *Tryporyza intact* larvae bore into sugarcane seedlings, causing a "dead heart" and reducing tiller numbers

(Li et al. 2024a, b). *Scirpophaga nivella* creates a distinctive "bunchy top" appearance in sugarcane (Surya Krishna et al. 2023).

The Mexican rice borer, *Eoreuma loftini*, is an invasive species that causes severe damage (Penn and Read 2024). The giant cane borer, *Telchin licus*, also inflicts significant damage in Central and South America (Li et al. 2024a, b). The African sugarcane stalk borer, *Eldana saccharina*, primarily attacks mature sugarcane and is considered the most destructive pest in South Africa (Mulcahy et al. 2023). The life cycle of these borers is crucial in understanding the extent of their damage to sugarcane (Joseph et al. 2024). Adult moths lay eggs on young sugarcane plants, and upon hatching, the larvae burrow into the stem or roots (Das and Rahman 2023). As they feed, they create visible tunnels and cavities that compromise the plant's structural integrity. These tunnels also provide entry points for pathogens, exacerbating the damage caused by the borers (Li et al. 2024a, b).

22.3 Injury and Economic Loss Caused by Borers

Yield losses caused by most borer species are primarily due to damage occurring before the formation of young shoots and reductions in stalk weight and juice quality after internode formation (Molter et al. 2023). The infestation index, which measures the percentage of bored internodes, is a reliable parameter for predicting these yield losses (Wang et al. 2024). However, some studies have also emphasized the impact of borer damage on juice quality (Priya et al. 2023). To assess the extent of damage, various methods of measurement have been employed for different borer species (Li et al. 2024a, b). For instance, *C. infuscatellus* can survive mild winters and attack ratooning seedlings, leading to a 28.6% incidence of "dead heart" (Li et al. 2024a, b), calculated as the ratio of damaged to total seedlings. In contrast, *C. venosatus* larvae feed inside the sugarcane stem, resulting in a 25% (w/w) production loss (Li et al. 2024a, b), which is determined by assessing the bored rate and estimating the sugar production loss.

In Brazil, during the 2010 and 2011 sugarcane growing seasons, *D. saccharalis* larvae bored an average of 25.77% and 19.01% of the internodes, respectively. In Louisiana, USA, during the 2018 plant cane and 2019 first ratoon trials, the percentage of bored internodes by *D. saccharalis* ranged from 3.7% to 27.7% (Li et al. 2024a, b). These studies illustrate the significant yield losses attributed to borer infestations, highlighting the variability in damage across regions and borer species.

Borer infestations significantly reduce sugarcane yield and sugar recovery, with the severity of losses closely linked to the extent of internode damage. For example, sucrose yield decreases substantially with increasing internode infestation, with estimated sugar yield losses of 8.83% and 19.80% per 1% of bored internodes in the first and second seasons, respectively (Shang et al. 2024). Furthermore, each 1% increase in bored internodes following *D. saccharalis* infestation is associated with an approximate 0.5% reduction in sugar yield per acre (Todd et al. 2024). In India, sugarcane yield losses due to *C. tumidicostalis* range from 8.2% to 12.6%, with

sucrose content losses ranging from 10.7% to 48.6% in Bihar (Li et al. 2024a, b). At the farmer level, these borers can cause cane yield losses of 8–10% and sugar recovery losses of 10–15% during processing (Li et al. 2024a, b).

In China, borers damage approximately 40% of the annual sugarcane crop, leading to yield losses between 10% and 25% (Khan et al. 2023). Studies there have shown that borer damage can result in cane losses of 2625–7950 kg/ha, accounting for 3.2–9.4% of cane production when the borer damage rate is between 5% and 20%, and 9960–13,537.5 kg/ha, or 11.7–15.9% when borer incidence ranges from 25% to 35% (Que et al. 2024). In the Xianggui sugarcane area of China, the average borer-damaged strain was 46.54%, leading to a yield loss of 14.89% and a sucrose content loss of 1.12% (Li et al. 2024a, b). The economic impact in China is also substantial, with agricultural losses estimated at 467 million yuan (about 60.31 million USD), industry losses reaching 856 million yuan (about 119.72 million USD), and national financial losses totaling 51 million yuan (Li et al. 2024a, b).

In Louisiana, USA, *D. saccharalis* damage was estimated to result in up to a 28% loss in cane weight per acre for a susceptible cultivar (CP 44–101) (Nikpay et al. 2023). The potential economic losses due to *E. loftini*, the Mexican rice borer, could reach up to 220 million USD if it becomes fully established in Louisiana (Nikpay et al. 2023). In Mexico, the crambid stalk borer *D. considerata* causes annual losses of up to ten million USD, while *E. loftini* damages 20% of sugarcane internodes, leading to losses of 575 USD/ha, with total annual losses estimated between 10 and 20 million USD (Li et al. 2024a, b). These figures reflect the significant economic burden caused by borer pests across multiple regions (Fig. 22.1).

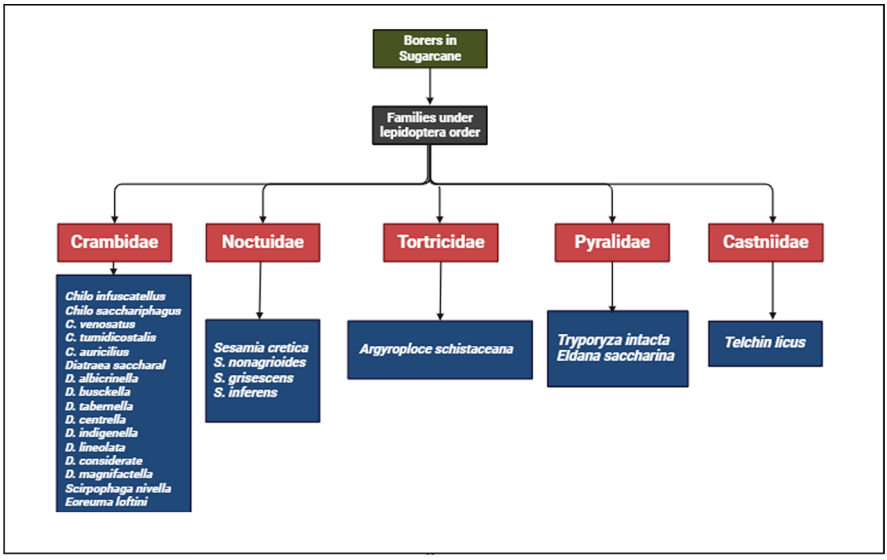

Fig. 22.1 Borer complex in sugarcane (Families under Lepidoptera order)

In Colombia's Cauca River Valley, economic losses attributed to *Diatraea* species are estimated at 143 kg of sucrose for each percent of bored internodes (Ángel-Salazar et al. 2023). In Papua New Guinea, *S. grisescens* caused 70% damage to commercial sugarcane plantings between January and June 1987, leading to an 18% loss in sugar production (Li et al. 2024a, b). In South Africa, *E. saccharina* causes a yield loss of 0.1% for every 1% of damaged stalks (Li et al. 2024a, b). In Indonesia, the economic losses caused by *S. nivella* can reach as much as 50% of sugarcane yields (Rex et al. 2023), while *C. sacchariphagus* and *C. auricilius* can damage up to 40% of stalks, resulting in a biomass yield reduction of 12.5 t/ha (19.2%) (Li et al. 2024a, b).

Furthermore, larvae of these borers damage sugarcane at all stages of plant development, and the entry holes they create not only allow for further damage from the borers but also provide pathways for secondary fungal infections, intensifying the overall crop impact (Fadda et al. 2024). On average, borer damage leads to a 10–30% reduction in sugarcane yield, corresponding to an annual loss of about 20% of the land allocated for sugarcane cultivation (Meena et al. 2020). These widespread economic losses highlight the critical need for novel technologies and strategies to mitigate borer damage in sugarcane-producing regions worldwide.

22.4 Mechanisms of Natural Resistance in Sugarcane

Sugarcane plants have evolved several natural mechanisms to resist borer attacks. These mechanisms can be broadly categorized into *physical defenses*, *chemical defenses*, and *behavioral responses*.

22.4.1 Mechanisms of Host-Plant Resistance

Pest and disease resistance refers to the inherent ability of an organism to reduce or eliminate the effects of pathogens, insects, or other harmful agents (Meena et al. 2020; Angon et al. 2023). Resistance is evident when a plant shows minimal or no symptoms, indicating that the pest struggles to spread or cannot spread at all on the host (Ntui et al. 2024). This resistant trait in plants typically results from specific morphological and biochemical characteristics that influence the behavior and biology of pests, thereby enhancing the plant's survival and productivity (Meena et al. 2020; Sruthi et al. 2024). The resistance level can be assessed by comparing resistant plants with more susceptible ones under similar conditions (Flors et al. 2024).

Plants can exhibit two types of natural pest resistance: induced and constitutive resistance (Gaddam et al. 2024). Induced resistance occurs when the plant detects an invader, prompting it to produce harmful proteins or metabolites that combat the pest (Malinowski et al. 2024). This type of resistance is triggered when the plant

recognizes specific molecules from the pest, such as proteins, fatty acid derivatives (e.g., fatty acid-amino acid conjugates), or other chemical compounds released by the pest (Kutty and Mishra 2023). Constitutive resistance, conversely, refers to the resistance already built into the plant, independent of pest attack. It can be morphological, structural, or chemical in nature (Meena et al. 2020; Poelman et al. 2023). Direct defenses immediately counteract herbivores attacking the plant, such as physical barriers (e.g., thorns, trichomes, waxes, and spines) and chemical defenses using secondary metabolites or specialized proteins (Lone et al. 2024). These defenses can be either constitutive or inducible. Indirect defenses, however, involve other species, such as natural predators of the pests, to help control pest populations (Ayoub et al. 2024). Plant resistance includes three main components: antixenosis, antibiosis, and tolerance. A resistant plant may display one or more of these mechanisms, but it is considered ideal for all three to be present in a highly resistant variety (Soujanya et al. 2024). Antixenosis involves traits that deter pests from settling on the plant, such as unappealing odors or textures. Antibiosis refers to the plant's ability to harm the pest's survival, growth, or reproduction through toxic substances. Tolerance refers to the plant's ability to withstand pest damage without significant loss in productivity, allowing it to recover despite the pest's presence.

22.4.2 Components of Host-Plant Resistance

Painter (1951) identified three key components of resistance, i.e., antixenosis, antibiosis, and tolerance, which can be physical, chemical, or a combination of both. These components are explained briefly below:

22.4.2.1 Antixenosis

Antixenosis is a resistance mechanism in plants that can be morphological, physical, or structural in nature, and it disrupts the behavior of insects, such as their ability to mate, lay eggs, or feed (Sahayaraj and Hassan 2023). This results in the insect avoiding the host plant. Factors like leaf color, texture, certain chemical compounds, or a combination of these characteristics can deter insect pests and prevent oviposition on the plant (Ayoub et al. 2024). Antixenosis has sometimes been used to develop resistant crop varieties (Ayoub et al. 2024). For example, in rice, resistance to *Cnaphalocrocis medinalis* (Lepidoptera: Crambidae) has been achieved by crossing a susceptible cultivar with a wild rice variety exhibiting antixenotic traits. *C. infuscatellus* prefers to lay eggs on 45-day-old plants in sugarcane, while older plants are avoided for oviposition. Research on the resistance mechanisms of two sugarcane varieties (R570 and R579) to *C. sacchariphagus* revealed that in cultivar R570, plants artificially infested with *C. sacchariphagus* showed a reduction in the number of larvae within 48 hours (Mahesh et al. 2024). Laboratory bioassays

confirmed that this reduction was due to antixenosis on the lower surface of the leaf sheath (Mahesh et al. 2024). The larvae showed susceptibility to antixenosis during the first, second, and third instar stages (Sahu et al. 2024). While antibiosis may have also played a role in resistance, the low number of dead larvae suggested that antixenosis was the primary resistance mechanism to *C. sacchariphagus* (Mahesh et al. 2024).

22.4.2.2 Antibiosis

Antibiosis negatively impacts the biology of insects attempting to use the plant as a host, and this effect can be either physical or chemical (Li et al. 2024a, b). The damage to the insect can be severe, particularly affecting larvae and eggs. Insects that survive antibiosis may experience long-term harm, such as stunted growth, slower development, and reduced fertility (Porcel et al. 2024). This resistance mechanism can be attributed to factors like allelochemicals, growth inhibitors, and morphological traits that prevent insect attacks (Angon et al. 2023). For example, allelochemicals such as glycoalkaloids in potatoes, γ-tomatine in tomatoes, gossypol in cotton, and rutin and chlorogenic acid in tomatoes are toxic to insects that feed on these plants (Angon et al. 2023). Compounds like amino acids, tannins, phenolics, lignins, and fiber content (ADF and NDF) have also been linked to sorghum's resistance to *Chilo partellus* (Porcel et al. 2024).

22.4.2.3 Tolerance

Tolerance enables plants to maintain normal growth and increase biomass despite insect infestations (Kumar et al. 2024). It is the most desirable resistance mechanism because it does not harm natural predator populations. In contrast, antixenosis and antibiosis can exert selective pressure on insect populations, potentially leading to the emergence of more aggressive biotypes and negatively affecting pest control strategies.

22.4.3 Role of Defense Mechanisms in Plants against Insect Pests

Various physical and chemical features on the plant surface play a key role in pest resistance (Nie et al. 2023), as they prevent insect pests from establishing themselves when they initially come into contact with the plant.

22.4.3.1 Physical Defenses/Barriers

The plant surface is the first point of contact for organisms attempting to establish themselves on the plant, so any changes or obstacles on the surface can contribute to pest resistance. Trichomes, or plant hairs, have created insect-resistant plant varieties (Rodríguez et al. 2024). These trichomes can be either nonglandular small hairs that physically deter insects or glandular, specialized structures that release chemical substances that can either be stored or vaporized on the plant's surface, helping to repel pests and prevent feeding (Kaur and Kariyat 2023). Recent studies suggest that trichomes may also play a role in the early detection of pests, as they become disturbed by the presence of insects, such as moths or larvae, which alerts the plant to the threat, enabling a faster response (Dixon and Gschwend 2024). The plant may then increase the density of trichomes on new leaves as part of its defense (Zhu et al. 2024).

Additionally, epicuticular waxes on the leaf surface help protect the plant by preventing dehydration and creating a slippery surface that makes it difficult for nonspecialized insects to settle on the plant (Kaur and Kariyat 2023). The composition of these waxes is crucial for determining resistance. In a study on *C. partellus*, it was found that the presence of leaf edge spines and surface waxes helps to redirect newly hatched larvae back towards the plant's stalk, preventing them from reaching the whorl (Kaur and Kariyat 2023). As a result, leaf surface waxes can contribute to the resistance levels of different plant varieties. Silicon, a plant mineral, can also enhance insect resistance, with studies showing that its use in fertilizers can increase resistance to pests like *E. saccharina* in sugarcane (Rodríguez et al. 2024). Leaf toughness is another crucial factor in pest resistance, as it can prevent insects from piercing and damaging plant tissues. While leaf toughness is generally seen as a physical trait, the chemical composition of the cell wall also contributes to this characteristic (Rodríguez et al. 2024).

22.4.3.2 Chemical Defenses

Chemical factors in plants contribute to resistance in two main ways: by reducing the nutritional value of the plant as a food source and by deterring insects through the production of toxic substances (Tlak Gajger and Dar 2021; Xu et al. 2023). Primary metabolites in plants, such as carbohydrates, amino acids, and lipids, are essential nutrients for insects, and the availability of these nutrients can influence the insect's lifespan, size, reproductive capacity, and mortality rates (Xu et al. 2023). Secondary metabolites, on the contrary, play a crucial role in plant defense mechanisms, including antixenosis, antibiosis, and tolerance (Haldhar et al. 2023). Phenolic compounds, a significant group of secondary metabolites, are essential in this defense process (Elshafie et al. 2023). These include coumarins, lignin, flavonoids, and tannins (Elshafie et al. 2023). Coumarins, found widely in plants, act as natural defense chemicals against insects, fungi, and bacteria (Rastija et al. 2023). Flavonoids, such as the C-glycosyl flavone maysin in maize silk, have been shown

to possess insecticidal properties, specifically against *Helicoverpa zea* (Lepidoptera: Noctuidae) (Haldhar et al. 2023).

Tannins can influence insect growth and development and serve as feeding deterrents for various animals. Terpenes, the largest class of secondary metabolites, form oils and resins that play a role in plant defense (Haldhar et al. 2023). Other nonprotein, nitrogen-containing secondary metabolites, such as alkaloids, cyanogenic glycosides, polyamines, phenylpropanoid conjugates (PPCs), and benzoxazinoids, also contribute to plant resistance (Haldhar et al. 2023). For example, dhurrin, a cyanogenic glycoside found in sugarcane leaves, is involved in constitutive and inducible resistance. The enzymes involved in its synthesis, such as IDP-glucose: phydroxymandelonitrile-O-glucosyltransferase and dhurrinase, become more active when the plant is attacked by the stalk borer *D. saccharalis* (Haldhar et al. 2023). Arginine, an essential amino acid in plants, also plays a role in defense by affecting insect nutrition when broken down by jasmonic acid-induced arginase. Benzoxazinoids found mainly in the Poaceae family (including maize, wheat, and sugarcane) are present in higher concentrations in young plants and in young tissues of older plants (Abdulfatah and Naji 2023). These compounds deter pests such as aphids and stalk borers, likely due to their antifeeding effects and their ability to hinder insects' ability to detoxify other plant defense chemicals (Abdulfatah and Naji 2023). While benzoxazinoids are typically constitutive, their levels can also increase as an induced response to insect attacks.

22.4.3.3 Protein-Based Defense

Insects possess four types of proteinases: serine, cysteine, aspartic acid, and metallo proteinases (Wang et al. 2023). Serine proteinases are present in Lepidoptera, while cysteine proteinases are found in Coleopteran insects (Zhiganov et al. 2024). Plant proteinase inhibitors (PIs) are part of the plant's natural defense against herbivores, and they are typically found in plant tissues that are more vulnerable to attack, such as bulbs, leaves, and seeds. These inhibitors can be constitutive or induced by wounding (Zhiganov et al. 2024). Research has shown that while proteinase inhibitors impact larval development, they do not cause death (Wang et al. 2023). The inhibitors likely affect the insect's digestive system by blocking the activity of midgut enzymes, reducing the availability of amino acids needed for protein synthesis. This, in turn, hinders the insect's growth, development, and reproduction (Wang et al. 2023). Alternatively, a compensatory response may occur, where the insect produces more digestive proteinases to offset the low levels of available amino acids, which results in a depletion of amino acids required for essential proteins. Studies using artificial diets with soybean proteinases have shown that sugarcane borers experience stunted growth and development (Wang et al. 2023).

Additionally, chitinase may help protect plants from insects by disrupting chitin-containing glycoproteins in the peritrophic matrix, a protective barrier in the insect gut that shields cells from digestive enzymes and pathogens. Lectins, sugar-binding proteins found primarily in legumes and other plants, also play a role in plant

defense. In sugarcane, lectin-like genes are expressed differently in various tissues, with higher expression in the leaf roll, apical meristem, and lateral buds and lower expression in the stalk (Wang et al. 2023). Polyphenol oxidase (PPO) enzymes are involved in the browning of plant tissues damaged by herbivory, wounding, or jasmonic acid (JA) signaling (Wang et al. 2023). PPOs are thought to help defend plants by producing o-quinones when insects feed on the plant. These quinones, formed through the interaction of PPO and phenolic compounds, modify free amino acids and sulfhydryl groups in the insect's mouth and gut. Additionally, when PPOs mix with phenolics in glandular trichomes, they create a sticky substance that traps small insects (Wang et al. 2023). In mesophyll tissue, PPOs cause protein modifications that reduce the digestibility of plant proteins in the insect's digestive system. This gene expression has been detected in sugarcane callus, seeds, root transition zones, and stalks.

22.4.3.4 Indirect Defenses

Recently, a new form of plant defense known as *the indirect defense* has been discovered, initially in maize, and has since been identified in several other plant species (Zhou et al. 2018). When insects attack plants, they release a complex mix of volatile compounds into the atmosphere from their vegetative parts, referred to as herbivore-induced plant volatiles (HIPVs) (Grunseich et al. 2020). These HIPVs can attract natural enemies of the herbivores, such as predators and parasitoids, known as constitutive indirect defense (Grunseich et al. 2020). Various types of HIPVs have been identified in plants, including alkenes, alkanes, and two jasmonates (cis-jasmone and methyl jasmonate). However, the primary compounds are C6 green leaf volatiles (GLVs), terpenes, and products derived from the shikimic acid pathway (Grunseich et al. 2020). These volatiles can defend the plant in different ways: by attracting herbivore enemies, directly deterring herbivores, or priming both the attacked plant and neighboring plants for more effective defense in the future (Zhou and Jander 2022). While indirect defense has been observed in natural hosts of *E. saccharina*, there are no known reports of sugarcane exhibiting this form of defense against the stalk borer (Misra et al. 2023). This absence may be due to the loss of this trait during sugarcane breeding, where only direct defense mechanisms have been actively selected.

22.5 Techniques

Initial screening of host-plant resistance should be conducted under controlled conditions, such as in a glasshouse or laboratory, to improve accuracy (Mookiah et al. 2021). Factors in the field, such as soil type, moisture levels, climate, and fluctuating pest populations, can decrease the precision of field trials (Hegedus and Maxwell 2022). Field assessments are typically carried out during the later stages of

sugarcane selection programs (Mahadevaiah et al. 2021). To effectively assess differences in resistance among sugarcane varieties, experiments should be designed to optimize conditions and consider the mechanisms of resistance involved (Mahadevaiah et al. 2021). Bioassays using insects on plants can evaluate induced and constitutive resistance, allowing for comparisons of insect populations, plant symptoms, and resistance components, such as antibiosis and antixenosis (Mahadevaiah et al. 2021). Several methods have been developed to assess sugarcane varieties for resistance to stalk borers, including measuring internode rind hardness, testing larval penetration into stalks, ovipositing tests with moths, controlled environment trials with artificial plant infestations, and incorporating leaf powders into artificial diets (Salgado et al. 2022).

To assess the resistance of maize varieties, black head stage egg masses and neonate larvae of *C. partellus* have been used for artificial infestations (Salgado et al. 2022). A camel hair paintbrush can also manually place larvae on plants for infestation (Salgado et al. 2022). It has been reported that using larvae for plant infestation is more effective than egg masses in host-plant resistance studies (Salgado et al. 2022). The plant's developmental stage is another vital consideration in resistance screening. However, studies have shown that even plants as young as 2 weeks old can differentiate between resistant and susceptible sugarcane varieties (Zhou and Jander 2022). The borer, *C. partellus* is known to begin attacking sorghum plants as early as 2 weeks after germination (Tamhane et al. 2021).

22.5.1 Use of Artificial Diet Bioassays

Artificial diets play a crucial role in arthropod research. Including leaf material in these diets can help identify constitutive resistance mechanisms in different plant varieties (Truzi et al. 2021). Using artificial diets in resistance screening is also beneficial for comparing sugarcane varieties under controlled conditions, as it eliminates environmental variations (Qamar et al. 2021). A large and sufficient supply of insects is necessary for successful resistance screening, along with an appropriate artificial diet to rear and maintain the insects used in these studies (Qamar et al. 2021). Several commercial diets have been developed to optimize insect growth and development by providing all the essential nutrients required for the target insect (Hawkey et al. 2021).

22.5.2 Engineering Sugarcane Against Pests

Sugarcane is an ideal candidate for genetic engineering due to its complex polyploidy, variable fertility, and genotype-environment interactions (Ali et al. 2024). The availability of high-frequency in vitro regeneration systems from various

explants makes sugarcane well-suited for genetic manipulation (Bidabadi and Jain 2020; Liang et al. 2024). Several genes related to disease and pest resistance, salt and drought tolerance, and sugar accumulation have been successfully introduced into sugarcane to enhance its traits (Nikpay et al. 2023). The success of producing transgenic sugarcane plants largely depends on the transformation method, the choice of target tissue or explant, and the tissue culture regeneration system used (Verma et al. 2023a, b). Different explants, such as axillary buds, apical meristems, immature inflorescences, and leaf segments, have all been used successfully to regenerate whole plants, demonstrating the wide range of totipotent tissues available for genetic transformation (Ali et al. 2024).

The global sugar industry faces significant economic losses (Li et al. 2024a, b; Chen et al. 2024) from insect pests and diseases, though exact quantification of pest damage is often lacking. While chemical control and integrated pest management (IPM) are standard approaches for managing insect pests, their effectiveness is usually limited due to practical challenges (Nikpay et al. 2023). The sugarcane canopy and its internal structure pose significant obstacles to applying chemical controls. Since borers' larvae enter the plant shortly after hatching, remain protected inside the leaf sheath or stem, and feed on stem tissues, they are challenging to target with insecticides. Moreover, systemic insecticides are largely ineffective due to poor movement within the plant. Alternative control measures, including mechanical methods, cultural practices, and biological agents, are often constrained by high costs and limited efficacy.

Sugarcane's genetic complexity and the lack of resistance genes in its germplasm have made conventional breeding for insect resistance challenging. However, genetic transformation and gene expression research advances have accelerated progress in engineering crops to protect against insect pests (Nikpay et al. 2023; Afghan et al. 2024). This includes the use of various molecules for pest control, such as proteinase inhibitors, plant lectins, ribosome-inactivating proteins, secondary plant metabolites, delta endotoxins, and vegetative insecticidal proteins from *Bacillus thuringiensis* (*Bt*) and related species. These molecules can be used alone or in combination with Bt genes for enhanced protection. Engineering insect resistance in sugarcane through the expression of these molecules is a promising approach to mitigate pest damage. The success of transgenic technology in improving insect resistance has been evident, particularly with stem borers (Kamatham et al. 2021). Insect resistance, often combined with herbicide resistance, currently ranks second in the global area occupied by biotech crops (Kamatham et al. 2021; Verma et al. 2024c).

22.5.3 Use Near-Infrared Reflectance Spectroscopy (NIRS) for Pest Detection

The analysis of plant components, such as proteins, carbohydrates, and lipids, is vital to many agricultural studies (Alves et al. 2021). However, chemical analyses can be time-consuming and expensive (Alves et al. 2021). In plant breeding trials, the need to analyze large numbers of samples often leads to the practice of bulk sampling, which may compromise the accuracy of experimental results (Swarup et al. 2021). Near-infrared spectroscopy (NIRS) has proven useful for overcoming these challenges. NIRS utilizes optical data based on the reflectance of a sample in relation to the amount of radiation it absorbs (Hong and Chia 2021). This technique operates within the near-infrared (NIR) region, typically between 750 and 2500 nm, adjacent to the mid-infrared region and the visible spectrum (Abbas et al. 2020). The bonds between atoms in plant tissues reflect the chemical composition of the tissue, and spectroscopy can be used to gather information about these bonds or the functional groups present (Abbas et al. 2020).

When a sample is exposed to irradiation, vibrations occur between the bonds, leading to stretching and bending. These vibrations produce a wave motion at frequencies specific to the functional group (Abbas et al. 2020). Absorption of incident light occurs when the frequency of the light matches the frequency of the bond vibrations, while light that does not match is reflected or transmitted. Bonds, such as C-H, O-H, S-H, and N-H, prevalent in plant tissue organic compounds, primarily absorb NIR light (Abbas et al. 2020). The chemical composition of the tissue determines the types and numbers of bonds, which in turn determine the wavelengths and amount of light absorbed. Therefore, the reflected light provides information about the chemical composition of the sample (Wang et al. 2023).

NIRS offers several advantages: it provides rapid and accurate analysis, is nondestructive to samples, does not require expensive or hazardous chemicals, can analyze large numbers of nonhomogeneous samples, and allows for multiple components to be assessed in a single measurement, which reduces costs (Abbas et al. 2020). However, there are some limitations to using NIRS. The initial cost of the equipment is high, and the instrument must be calibrated for each component to be analyzed (Abbas et al. 2020). Despite this, portable NIRS instruments have become widely used, particularly for measuring nitrogen content in leaves to optimize fertilizer applications. These portable devices can scan the entire NIR spectrum and often use fiber optic probes (Abbas et al. 2020). NIRS spectra can be complex due to overtones and combinations resulting from light scattering and absorptions in the mid-infrared region (Abbas et al. 2020). This complexity makes direct interpretation of the NIR spectrum challenging, as few absorption bands correspond to only one functional group. To address this, statistical models must correlate specific absorptions with laboratory assays for particular constituents in a range of tissue samples. As a result, NIRS is considered a secondary method for determining chemical composition, relying on comparisons with spectra from

samples whose composition has been determined using traditional laboratory techniques (Abbas et al. 2020).

22.6 Breeding for Borer Resistance in Sugarcane: An Overview

22.6.1 Traditional Breeding Approaches

Traditional breeding for managing borer pests in sugarcane focuses on developing cultivars with inherent resistance to a wide range of borer species, critical pests in many sugarcane-growing regions (Viswanathan et al. 2022). These borers, including those from genera, such as *Chilo*, *Diatraea*, *Sesamia*, and others, cause significant economic losses by damaging sugarcane's structural integrity and reducing both yield and sugar content (Dwivedi et al. 2022; Tanveer et al. 2024). Traditional breeding approaches aim to create sugarcane varieties that exhibit either natural resistance or tolerance to these pests, while also maintaining or enhancing agronomic traits like yield, disease resistance, and overall crop vigor (Dwivedi et al. 2022).

22.6.1.1 Screening for Resistance

Screening for natural resistance is a foundational step in traditional breeding for borer management (Biselli et al. 2022; Tanveer et al. 2024). This involves identifying sugarcane varieties that are less susceptible to pest infestation and damage. Screening is done through:

- *Field trials*: Sugarcane varieties are grown in pest-prone areas, and the extent of borer damage (e.g., tunneling, dead-heart symptoms, and stalk damage) is assessed (Nhamo and Talabi 2023). Plants that show minimal damage are considered for breeding purposes.
- *Laboratory tests*: Some research programs also use controlled environments to study borer behavior and test different varieties' resistance to larval feeding or egg-laying preferences (Nhamo and Talabi 2023).

Key Screening Traits

- *Physical traits*: Thicker stems, more inflexible leaf tissues, and more rigid cell walls can deter borers from penetrating and feeding on the plant (Bojović et al. 2024).
- *Chemical defenses:* Certain plant varieties may produce secondary metabolites like alkaloids or phenolic compounds, reducing the plant's attractiveness to pests or making it toxic to larvae (Divekar et al. 2022).

- *Growth stage*: Screening can also be done at different growth stages (e.g., early shoot, internode, and mature cane) to identify resistance in specific stages that are most vulnerable to attack (Nikpay et al. 2023).

22.6.1.2 Hybridization and Crossbreeding

Hybridization between resistant and high-yielding cultivars is the most common method to incorporate pest resistance into commercial sugarcane varieties. The process typically follows these steps:

- *Initial crossbreeding*: Resistance is often introduced from wild or less-agronomic sugarcane species into high-yielding varieties (Hussain et al. 2024). For instance, resistance genes from wild relatives or older cultivars are crossed with newer, commercially viable cultivars (Perera et al. 2022).
- *Backcrossing*: After the initial cross, the progeny are backcrossed with the parent variety that exhibits desirable agronomic traits (e.g., high yield or disease resistance) to ensure that the resulting cultivar retains these characteristics while incorporating pest resistance (Begna 2021).
- *Selection of hybrids*: Throughout the backcrossing process, hybrids showing the best combination of pest resistance, high yield, and other desirable agronomic traits (such as disease resistance, drought tolerance, or high sucrose content) are selected for further evaluation (Yadav et al. 2020).

22.6.1.3 Resistance Mechanisms in Breeding

Sugarcane resistance to borers can be achieved through various genetic mechanisms, and breeders aim to select varieties that express one or more of these mechanisms:

- *Physical resistance*:
  - *More inflexible stem tissues*: Varieties with harder or thicker stems are less penetrable to borers. This can prevent larvae from burrowing into the plant (Dlamini 2021).
  - *Leaf toughness*: Stronger leaves may be more difficult for borers to feed on, particularly for species like *Chilo,* which attack young shoots (Dwivedi et al. 2022).
- *Chemical resistance*:
  - *Antifeedant compounds*: Some sugarcane varieties produce chemicals that deter feeding, making them less attractive to borer larvae. For example, specific plant metabolites can make the plant tissue taste unpalatable or toxic to larvae (Tlak Gajger and Dar 2021).

  - *Insecticidal compounds*: Some sugarcane varieties may naturally produce insecticidal compounds that reduce the survival of borer larvae. These compounds could include toxins like protease inhibitors or alkaloids, which disrupt the larvae's digestive processes (Wahengbam et al. 2021).

- *Behavioral resistance*:
- Some sugarcane varieties may affect the behavior of borers. For example, some plants may produce volatile compounds that deter moths from laying eggs or attract natural predators of the borers (Jacob et al. 2021).

22.6.1.4 Breeding for Tolerance Versus Resistance

Resistance refers to a plant's capacity to prevent damage from pests; whereas, tolerance describes its ability to withstand pest damage without experiencing substantial yield loss (Matova et al. 2022; Bass and Nauen 2023). Breeding for tolerance is essential for managing borers, particularly when developing varieties for regions with high pest pressure (Galluzzi et al. 2020). Tolerance breeding may also focus on enhancing plant vigor to offset the adverse effects of borer damage (Wani et al. 2022).

Tolerance traits might include:

- *Regrowth after damage*: Some sugarcane plants can compensate for borer-induced damage by quickly regrowing new shoots or stems, minimizing yield loss (Galluzzi et al. 2020).
- *Delayed impact on yield*: Varieties that can withstand early-stage borer infestations may still produce good yields at harvest, even if the borers affect the crop in the early growth stages (Galluzzi et al. 2020).

22.6.1.5 Polygenic Resistance

Polygenic resistance involves the contribution of multiple genes to resist pest attacks (Badji et al. 2020). Borers, such as those in the *Chilo*, *Diatraea*, and *Sesamia* genera, are often controlled by a complex array of genes, which makes breeding for resistance a long-term effort (Soujanya et al. 2024).

- *Multiple genes for resistance*: Since borer pests can overcome single-gene resistance over time, breeding programs often focus on pyramiding multiple resistance genes from different sources to develop more challenging cultivars for pests to overcome (Budeguer et al. 2021).
- *Gene stacking*: Besides pyramiding resistance genes, breeders may also stack genes that confer resistance to different types of borers (Salim et al. 2020). For example, a cultivar might be resistant to both the early shoot borer (*C. infuscatellus*) and the stalk borer (*C. auricilius*) by incorporating resistance traits from different genetic sources (Nikpay et al. 2020).

22.6.1.6 Selection for Reduced Pest Suitability

In addition to direct resistance mechanisms, breeders may select traits that reduce pest suitability (Miedaner and Juroszek 2021). These include:

- *Reduced susceptibility to egg-laying*: Some sugarcane plants may produce fewer pheromones or volatile organic compounds that attract moths, reducing the likelihood of egg-laying (Bakthatvatsalam et al. 2022).
- *Nonpreferred feeding sites*: By selecting plants with fewer or less attractive feeding sites (e.g., more compact or more fibrous tissue), breeders can reduce the likelihood of pest infestation (Thiel et al. 2021).

22.6.1.7 Field Evaluation and Phenotyping

After developing new resistant varieties through crossbreeding, extensive field evaluations are conducted to assess the actual effectiveness of resistance traits in real-world conditions. This includes:

- *Field resistance trials*: Sugarcane varieties are planted in pest-prone areas, and breeders assess the level of pest damage under natural field conditions (Bakthatvatsalam et al. 2022).
- *Quantifying pest damage*: Damage assessment might include the number of larvae per plant, the extent of tunneling, and reductions in yield or sugar content (Miedaner and Juroszek 2021).
- *Long-term trials*: Given that pest pressures can vary yearly, long-term trials help identify varieties that remain resistant to borers across multiple growing seasons (Miedaner and Juroszek 2021).

22.6.1.8 Integrated Pest Management (IPM)

While traditional breeding focuses on developing resistant varieties, Integrated Pest Management (IPM) approaches are often integrated with breeding efforts. IPM integrates various control strategies to manage borer pests more effectively:

- *Cultural practices*: Crop rotation, soil management, and timely planting can help reduce borer populations (Jasrotia et al. 2023).
- *Biological control*: Natural predators of sugarcane borers, such as parasitic wasps, can be encouraged in the field (Maurya et al. 2022).
- *Chemical control*: In some cases, chemical insecticides may still be necessary, but these are used with resistant varieties to minimize their use (Helps et al. 2020).

22.6.1.9 Challenges in Traditional Breeding for Borer Management

- *Long breeding cycles*: Developing new borer-resistant sugarcane varieties can take many years, as it involves multiple generations of crossbreeding, selection, and testing (Hong and Huang 2024).
- *Complex pest biology*: Borer pests exhibit complex biology, with multiple species targeting different growth stages of sugarcane. Developing broad-spectrum resistance can be challenging (Hong and Huang 2024).
- *Pest adaptation*: Over time, pests may adapt to resistant varieties, requiring continuous monitoring and the development of new resistance sources.
- *Maintaining high yields*: Resistance traits must be incorporated without sacrificing the high yields, quality, or other desirable agronomic traits of the sugarcane variety (Hong and Huang 2024).

22.6.2 Innovative Breeding Techniques for Enhancing Borer Resistance

22.6.2.1 Marker-assisted selection (MAS) and Its Role in Identifying Resistant Traits

Molecular Marker-Assisted Selection (MAS) has become a groundbreaking and influential method for speeding up the development of cultivars resistant to borers (Lu et al. 2024). This approach utilizes molecular biology and genomics to detect and select plants with genetic markers linked to beneficial traits, such as pest resistance (Salgotra and Stewart Jr 2020).

22.6.2.1.1 Understanding Molecular Marker-Assisted Selection (MAS)

What Is MAS?

Molecular Marker-Assisted Selection (MAS) is a breeding method that utilizes molecular markers with specific DNA sequences linked to desired traits to identify and select individuals with those traits early on, often before they are visibly expressed in the plant (Kumawat et al. 2020; Hussain et al. 2024). In the case of sugarcane and borer resistance, MAS enables breeders to detect plants with genetic markers associated with borer resistance, even before the plants are exposed to pest pressures in the field (Cursi et al. 2022; Lu et al. 2024).

Key Components of MAS

1. *Molecular markers*: These are specific DNA sequences closely associated with a trait of interest, such as pest resistance. They do not necessarily cause the trait but are located near genes (Salgotra and Chauhan 2023).

2. *Trait linkage*: Through genetic mapping of plants, breeders can pinpoint markers reliably passed down along with resistance traits (Salgotra and Stewart Jr 2020).
3. *Selection*: Breeders can use the markers to select the best candidates for further breeding or commercialization without waiting for phenotypic traits (such as borer resistance) to manifest (Allier et al. 2020).

How Does MAS Work?

- *Identification of Resistance Genes*: The initial step in MAS is identifying the genes responsible for borer resistance. This involves understanding the genetic foundations of pest resistance in sugarcane and mapping the genes that govern these traits. Resistance genes may include those that affect the production of defensive chemicals (e.g., phenolic compounds) or traits that make the plant physically challenging for pests to feed on, such as reinforced cell walls (You et al. 2021).
- *Marker Development*: After identifying the resistance genes, scientists create molecular markers [e.g., simple sequence repeats (SSRs), single nucleotide polymorphisms (SNPs), or amplified fragment length polymorphisms (AFLPs)] that are closely associated with these genes. These markers can then be used to monitor future generations' inheritance of resistance traits (Hasan et al. 2021).
- *Genotyping and Selection*: In the breeding program, sugarcane plants are genotyped to detect the molecular markers linked to borer resistance. This genotyping uses high-throughput methods like PCR (Polymerase Chain Reaction) and sequencing. Plants with the desired markers are then chosen for continued breeding (Subekti et al. 2020).
- *Backcrossing and Final Selection*: Plants that carry the resistance markers are then backcrossed with a high-yielding or commercially valuable parent to maintain key agronomic traits (such as high yield, disease resistance, and sugar content) while incorporating pest resistance. Over multiple generations, breeders continue to select plants that exhibit both borer resistance and favorable agronomic characteristics (Subekti et al. 2020).

22.6.2.1.2 Advantages of MAS for Borer Resistance in Sugarcane

MAS provides several benefits over conventional breeding techniques, particularly regarding pest management and enhancing sugarcane varieties for borer resistance.

Faster and more Accurate Selection

Traditional breeding involves phenotypic screening, where breeders visually assess the resistance of plants to pests over multiple growing seasons. This can take years, particularly in perennial crops like sugarcane, which have long breeding cycles. MAS enables breeders to select for borer resistance early in the breeding cycle, even

at the seedling stage, by identifying plants with resistance markers before they are exposed to pest pressure (Souza et al. 2021).

- *Accelerated Breeding Cycle*: MAS shortens the time required to incorporate resistance traits into commercial varieties by allowing for early identification and selection of resistant plants (Souza et al. 2021).
- *Higher Precision*: MAS improves selection accuracy by allowing breeders to focus on genetic markers linked to resistance rather than relying solely on observable traits that can be influenced by environmental factors (Souza et al. 2021).

Reduced Risk of Environmental and Pest Resistance Variation

One of the challenges in managing borer pests is the variation in pest populations from season to season. Traditional phenotypic screening may fail to account for pest population dynamics and environmental factors influencing pest outbreaks. MAS, on the other hand, allows breeders to focus on genetic markers linked to resistance, regardless of seasonal pest variations.

- *Consistent Selection*: MAS reduces the influence of environmental variables and ensures that resistance traits are consistently passed down, even in the face of changing pest populations (Ma et al. 2021).
- *Resistance to Multiple Pests*: By using MAS, breeders can select for resistance to multiple borer species simultaneously, ensuring that the final cultivar is resilient against a broad spectrum of pests (Ma et al. 2021).

Reduced Dependency on Chemical Pesticides

One of the primary benefits of breeding for borer resistance is reducing the need for chemical insecticides. By developing varieties with inherent borer resistance, MAS can help reduce pesticide use, leading to more environmentally sustainable farming practices.

- *Eco-friendly Pest Management*: By focusing on genetic resistance, MAS reduces the dependency on chemical pesticides, leading to less environmental pollution, healthier ecosystems, and lower production costs for farmers (Allier et al. 2020).

Improved Resistance Durability

Traditional pest management methods, including chemical control, often result in the evolution of pest resistance. Over time, pests can develop resistance to specific pesticides, leading to diminished control. MAS, however, offers a way to develop more durable resistance by incorporating complex, multigene resistance mechanisms that are less likely to be overcome by pest adaptation.

- *Incorporation of Multiple Resistance Mechanisms*: MAS enables the integration of multiple resistance genes into a single cultivar, lowering the likelihood of pests developing resistance to just one defense mechanism (Allier et al. 2020).

22.6.2.1.3 Challenges of MAS in Borer Resistance Breeding

Despite the clear advantages of MAS, some challenges need to be addressed to maximize its effectiveness in sugarcane breeding programs.

Limited Knowledge of Resistance Mechanisms

Although substantial progress has been made in understanding the genetic basis of pest resistance, the mechanisms underlying borer resistance in sugarcane are still not completely understood. Many resistance traits are likely polygenic (involving multiple genes), making it difficult to pinpoint specific markers linked to resistance (Subekti et al. 2020).

- *Complex Resistance Mechanisms*: Borer resistance in sugarcane is likely to involve a combination of traits, such as physical defenses (e.g., stem toughness) and chemical defenses (e.g., production of antifeedant compounds). The complexity of these mechanisms makes it challenging to identify markers for MAS (Subekti et al. 2020).
- *Marker Availability*: For MAS to be effective, suitable molecular markers tightly linked to resistance genes must be available. In many cases, these markers may not yet be discovered or characterized (Subekti et al. 2020).

Incomplete Genomic Resources

The genetic resources for sugarcane are less advanced than other major crops, such as maize, rice, or wheat. The sugarcane genome is large and complex, complicating efforts to identify all relevant genetic markers for pest resistance(Miedaner and Juroszek 2021).

- *Large and Complex Genome*: Sugarcane's polyploid genome makes it more challenging to identify specific markers associated with resistance traits. This requires extensive genomic research and the development of high-quality genetic maps(Miedaner and Juroszek 2021).
- *Lack of Comprehensive Genetic Databases*: For MAS to be effective, comprehensive genetic databases must link molecular markers with traits. Developing these sugarcane databases is ongoing (Miedaner and Juroszek 2021).

Integration with Other Breeding Strategies

- While MAS is a powerful tool, it cannot be used in isolation. Combining MAS with other breeding techniques, such as conventional phenotypic selection, genomic selection (GS), and transgenic approaches, is essential for developing high-yielding, borer-resistant sugarcane varieties.
- *Multipronged Approach*: MAS works best with other strategies, such as genomic selection, transgenic breeding, and advanced phenotypic evaluations.

A well-integrated breeding program will be more effective at addressing the challenges posed by borer pests.

22.6.2.1.4 Future Prospects of MAS for Borer Resistance in Sugarcane

The future of MAS in sugarcane breeding for borer resistance looks promising, particularly as advances in genomics, biotechnology, and molecular tools continue to accelerate. The following trends will likely shape the future of MAS in this field:

- *Enhanced Marker Development*: As genomic resources improve and more sugarcane-specific markers are identified, MAS will become more effective and accessible. Emerging high-throughput technologies, like next-generation sequencing, will facilitate markers' faster and more precise development (Miedaner and Juroszek 2021).
- *Polygenic Resistance*: As researchers identify more markers associated with the multiple genes responsible for borer resistance, the ability to select for complex, polygenic resistance will improve. This progress will enable the development of sugarcane varieties with more durable and broad-spectrum resistance (Allier et al. 2020).
- **Integrated Pest Management**: MAS will likely be part of a broader Integrated Pest Management strategy, combining genetic resistance with cultural practices, biological control, and reasonable pesticide use to manage borer pests more sustainably and holistically (Allier et al. 2020).

22.6.3 Genetic Modification (GM) and Gene Editing in Sugarcane for Pest Resistance: Innovations and Advancements: An Overview

Sugarcane, one of the world's most important crops, faces numerous biotic challenges, particularly from borers, which cause substantial yield losses. Traditional breeding methods have long been employed to enhance resistance to pests in the context of crop improvement. However, the emergence of genetic modification (GM) and gene editing technologies, such as CRISPR-Cas9 and RNA interference (RNAi), have revolutionized how researchers approach pest resistance in crops like sugarcane (Tyagi et al. 2020). These cutting-edge tools promise to accelerate the development of pest-resistant varieties, offering significant advantages over conventional methods. In this context, we explore how these technologies work, their potential applications in sugarcane pest resistance, and the innovations they bring to the field (Tyagi et al. 2020). Genome editing (GE), which involves inserting or deleting DNA in an organism, is a key technique for genetic modification (Miedaner and Juroszek 2021). There are four leading nuclease families used in GE: meganucleases, transcription activator-like effector nucleases (TALEN), zinc-finger

nucleases (ZFN), and CRISPR/Cas9 technologies (Miedaner and Juroszek 2021). CRISPR/Cas9, originally part of the adaptive immune system of prokaryotes, defends against viruses by integrating short sequences (spacers) from past infections into the CRISPR locus. These integrated sequences, called crRNAs, guide Cas nucleases to target and cut invading DNA during reinfection. The discovery of CRISPR/Cas9's ability to programmatically target DNA opened new possibilities for genome editing (Miedaner and Juroszek 2021).

While CRISPR/Cas9 is the most widely used genome-editing tool today, TALEN-mediated GE has also shown promise in sugarcane, particularly for enhancing bioethanol production (Shabbir et al. 2021). However, sugarcane, with its large genome size (approximately 10 GB) and multiple homologous gene copies (8–12), presents significant challenges in genome editing (Shabbir et al. 2021). One key obstacle in sugarcane molecular improvement is the issue of transgene silencing at both the transcriptional and posttranscriptional levels (Shabbir et al. 2021).

To mitigate these challenges, efficient promoters can be used to regulate GE tools like Cas9. However, like other GE nucleases, Cas9 can cause off-target mutations, cleaving unintended DNA sequences (Tyagi et al. 2020). Selecting specific promoters and truncated gRNA-Cas9 complexes can help minimize off-target effects while achieving high mutation rates (Shabbir et al. 2021). Moreover, developing modified Cas9 variants to address off-target issues is another avenue for improving GE efficiency (Shabbir et al. 2021).

While there are still challenges to overcome, the use of CRISPR-associated nucleases, including different Cas9 variants, holds great potential for successful genome editing in sugarcane and other complex polyploid crops.

22.6.3.1 Genetic Modification in Sugarcane for Pest Resistance

Genetic modification (GM) involves introducing specific genes into a plant's genome to provide new traits, such as resistance to pests. In sugarcane, GM has been utilized to enhance resistance to borer pests, which are known to cause significant damage to the crop.

The most common method involves inserting genes from other organisms, such as bacteria or other plants, into the sugarcane genome to enhance its defensive traits (Dlamini 2021).

22.6.3.1.1 Bt Sugarcane: A Key GM Innovation

One of the most notable examples of genetic modification in sugarcane is the development of *Bt* (*Bacillus thuringiensis*) sugarcane. The *Bt* gene, derived from the soil bacterium *Bacillus thuringiensis*, produces toxic proteins for certain insect pests. When inserted into sugarcane, these proteins can specifically target and kill borers and other harmful insects without affecting beneficial species or the environment (Shabbir et al. 2021).

The development of Bt sugarcane offers several advantages over traditional pest control methods:

- *Reduced Chemical Use*: *Bt* sugarcane minimizes the need for chemical insecticides, leading to more eco-friendly farming practices (Shabbir et al. 2021).
- *Sustained Resistance*: As *Bt* proteins target specific pests, the resistance is more direct and often longer-lasting compared to conventional pesticides, which can lose effectiveness as pests evolve resistance (Shabbir et al. 2021).
- *Lower Production Costs*: Reduced pesticide use translates into lower input costs for farmers (Shabbir et al. 2021).

Despite its potential, GM sugarcane faces several regulatory hurdles and public concerns related to genetic modification. The approval process for GM crops is often lengthy and complex, and issues like gene flow to non-GM crops and consumer acceptance, remain significant challenges. Nevertheless, the potential for GM technology to reduce the dependency on chemical pesticides and to provide more targeted and sustainable pest control solutions remains promising (Cursi et al. 2022).

22.6.3.2 Gene Editing Technologies: CRISPR and RNAi in Pest Resistance

While genetic modification (GM) has proven to be a powerful tool for crop improvement, gene editing technologies like CRISPR-Cas9 and RNA interference (RNAi), offer greater precision and flexibility in manipulating plant genomes. These techniques allow for the direct modification of specific genes without introducing foreign DNA, making them more favorable to regulatory authorities and consumers (Iqbal et al. 2021).

22.6.3.2.1 CRISPR-Cas9: Precision Gene Editing

CRISPR-Cas9 has revolutionized genetic research by enabling precise edits to the genome of organisms, including plants. The technology operates by utilizing a guide RNA molecule that directs the Cas9 protein to a specific location in the genome, where it makes a precise cut in the DNA.

This allows for the targeted addition, deletion, or alteration of genes with remarkable accuracy (Zhao et al. 2021). Specific and targeted genetic changes, CRISPR has the potential to speed up the development of pest-resistant sugarcane varieties, providing a degree of precision that was not possible with conventional genetic modification methods (Amaresh et al. 2024).

In sugarcane, CRISPR could be used to:

- *Knockout Susceptibility Genes*: By editing the genes responsible for sugarcane's susceptibility to borers, researchers can make the plants more resistant to pest

attacks. For example, genes that produce certain enzymes or proteins required for the pests to feed could be disrupted, making the sugarcane less palatable or harmful to the pests (Zhao et al. 2021).
- *Enhance Native Defense Mechanisms*: CRISPR could be used to enhance the natural defense mechanisms of sugarcane. For instance, researchers could activate the plant's ability to produce antifeedant compounds or reinforce physical defenses like thicker cell walls that are more difficult for borers to penetrate (Zhao et al. 2021).

22.6.3.2.2 RNAi Technology: Silencing Pest Genes

RNAi operates by silencing or reducing the expression of specific genes in the pest rather than altering the plant itself. In the case of sugarcane, RNAi can be engineered to target genes in borer pests that are essential for their survival or reproduction (Rajam 2020).

For instance, RNAi can be used to:

- *Target Borer-Specific Genes*: RNAi can disrupt crucial genes involved in the development or feeding of borers, effectively preventing them from causing damage. This approach is highly targeted and does not threaten other organisms (Rajam 2020).
- *Reduce Pest Fertility*: RNAi can also be used to target genes that are essential for pest reproduction, potentially reducing pest populations over time without relying on pesticides (Rajam 2020; Arya et al. 2021).

One of the most exciting aspects of RNAi is that it can be applied in a more environmentally friendly manner. Instead of introducing a foreign protein into the plant (as with GM), RNAi involves the expression of plant-derived RNA molecules that trigger gene silencing in pests, making it a less controversial and more sustainable alternative to chemical control methods (Arya et al. 2021).

22.6.3.3 Innovation and Future Directions

Applying genetic modification and gene editing technologies in sugarcane breeding for pest resistance represents a significant leap forward in agricultural biotechnology. These innovations promise to improve sugarcane productivity by reducing pest damage and offer environmental and economic benefits by reducing the reliance on chemical pesticides (Salgotra and Stewart Jr 2020).

As gene editing techniques, such as CRISPR and RNAi, become refined, their potential to create more sustainable and resilient crops expands. The ability to:

- *Develop Multiresistant Varieties*: By integrating GM and gene editing technologies, it is possible to create sugarcane varieties that are resistant to multiple spe-

cies of borers at once. This multitrait strategy enhances the resilience of sugarcane crops, helping them withstand the challenges posed by evolving pest populations (Arya et al. 2021).
- *Speed Up Breeding Cycles*: Gene editing accelerates the breeding process by allowing precise modifications without requiring long breeding cycles. This is especially important for perennial crops like sugarcane, where traditional breeding can take years.

In the future, integrated pest management strategies that combine GM, gene editing, and MAS will likely become the standard. These technologies could be used together to create sugarcane varieties with enhanced resistance, better yield potential, and improved sustainability, thus addressing both environmental and economic challenges in the sugarcane industry.

22.6.4 Transgenic Approaches and their Potential in Sugarcane

22.6.4.1 What Are Transgenic Approaches?

Transgenic plants have been genetically modified using various techniques by introducing foreign DNA into their genome. The inserted genes may come from a different species (inter-species transgenes) or even within the same species (intra-species) (Dinesh Babu et al. 2022). In the case of sugarcane, the most common transgenic techniques involve:

- *Gene insertion* using *Agrobacterium* or particle bombardment methods.
- *Agrobacterium*-mediated transformation, where the bacterium introduces specific genes into the sugarcane plant cells (Dinesh Babu et al. 2022).
- *Gene gun (biolistic) technology*, where DNA-coated particles are shot into the plant cells (Dinesh Babu et al. 2022).

Once the foreign genes are successfully incorporated into the plant's DNA, they can express traits, such as pest resistance, improved yield, or tolerance to environmental stress. Transgenic sugarcane has the potential to address several challenges that traditional breeding methods struggle to resolve efficiently (Dinesh Babu et al. 2022).

22.6.4.2 Key Potential Applications of Transgenic Approaches in Sugarcane

22.6.4.2.1 Pest Resistance

One of the key advantages of transgenic sugarcane is the ability to develop varieties that are resistant to pests, especially borer insects, which are a significant contributor to yield losses in sugarcane crops. Traditional pest control often relies on chemical pesticides, which can be costly, environmentally harmful, and may lead to pesticide resistance in pest populations. Transgenic approaches can provide more sustainable and effective solutions (Babu et al. 2021).

- *Bt* Sugarcane: The most well-known example of transgenic pest resistance is the incorporation of the *Bacillus thuringiensis* (*Bt*) gene, which produces a protein toxic to specific insect pests. *Bt* sugarcane is genetically modified to produce the Bt protein, which provides inherent resistance to borers and other insect pests. This built-in pest resistance can significantly reduce the reliance on chemical insecticides, lower production costs, and decrease environmental impact (Babu et al. 2021).
- *Other Resistance Traits*: Beyond Bt, different genes can be inserted into sugarcane to provide resistance against various pests. These include genes for the production of toxins, insect growth regulators, or proteins that make the plant physically more challenging for insects to consume (Babu et al. 2021).

22.6.4.2.2 Enhanced Sugar Yield and Quality

Genetic modifications can also be aimed at improving the productivity and quality of sugarcane. Transgenic approaches can increase sugar accumulation in the cane, enhance the efficiency of photosynthesis, or alter the biochemical composition of the plant to produce higher-quality sugar or even biofuels (Rosa et al. 2022; Joshi et al. 2022, 2023).

- *Improved Sugar Content*: By targeting genes involved in sucrose biosynthesis and sugar transport, transgenic sugarcane can be developed to produce higher sugar yields per hectare, benefiting both commercial sugar production and biofuel industries (Rosa et al. 2022).
- *Improved Biomass Production*: Modifying sugarcane to increase its biomass production and enhance its fiber content can improve its utility as a bioenergy crop, helping meet the growing demand for renewable energy (Rosa et al. 2022).

22.6.4.3 Challenges and Future Outlook

Despite the promising potential of transgenic sugarcane, there are several challenges associated with its development and commercialization:

- *Regulatory Hurdles*: Transgenic crops face rigorous regulatory approval processes, varying significantly between countries. These regulations are designed to evaluate the safety of transgenic plants concerning human health, animal welfare, and environmental impact (Budeguer et al. 2021; Hawkey et al. 2021).
- *Public Perception*: There is ongoing debate regarding the safety and ethics of genetically modified organisms (GMOs), particularly in regions where GM crops are not widely accepted. Public resistance to GMOs can challenge their widespread adoption (You et al. 2021).
- *Gene Flow and Environmental Risks*: There is a potential risk of gene flow between transgenic sugarcane and wild relatives or non-GMO crops, which could lead to unintended ecological consequences (You et al. 2021).

Despite the challenges, the potential advantages of transgenic, especially in terms of pest and disease resistance, enhanced stress tolerance, and increased indicate that this technology will be a key component of future sugarcane breeding programs. Ongoing research and technological advancements will unlock new opportunities for transgenic sugarcane to meet global food, biofuel, and environmental needs (You et al. 2021).

22.6.5 The Role of Wild Relatives in Enhancing Pest Resistance in Sugarcane

Sugarcane (*Saccharum* spp.) is a key global crop for sugar production, biofuels, and other industrial applications. However, it faces numerous challenges, particularly from pests, diseases, and environmental stresses, limiting productivity and quality. To address these challenges, breeders have increasingly turned to wild relatives of sugarcane as a valuable source of genetic materials to enhance resistance traits. Wild relatives, often overlooked in traditional breeding programs, possess a wealth of untapped genetic diversity that can be harnessed to improve sugarcane's resilience and adaptability to biotic and abiotic stresses. This approach is gaining momentum as it offers a sustainable way to develop more resistant, higher-yielding, and climate-resilient sugarcane varieties (Viswanathan et al. 2022).

22.6.5.1 Understanding Wild Relatives of Sugarcane

Wild relatives of sugarcane belong to the genus *Saccharum* and other genera closely related to sugarcane, such as *Erianthus*, *Miscanthus*, and *Sorghum*. These wild species are typically found in the tropical and subtropical regions of Asia, Africa, and Oceania. They possess a variety of traits that are either absent or less developed in cultivated sugarcane, including resistance to pests and diseases, tolerance to drought and salinity, as well as improved resilience to environmental stresses, such as cold temperatures and high soil salinity (Dinesh Babu et al. 2022).

The most important wild relatives for sugarcane breeding include:

- *Saccharum spontaneum:* A wild species known for its exceptional resistance to drought, salinity, and pests (Dinesh Babu et al. 2022).
- *Erianthus arundinaceus*: A grass species that exhibits traits such as cold tolerance, resistance to biotic stresses, and high biomass yield potential (Meena et al. 2020; Dinesh Babu et al. 2022).
- *Miscanthus* spp: Closely related to sugarcane, these plants are used in bioenergy research and exhibit resilience to harsh growing conditions (Dinesh Babu et al. 2022).
- *Sorghum bicolor*: Although not a direct relative of sugarcane, sorghum shares a common ancestor with sugarcane and is widely used as a model for drought and pest resistance (Dinesh Babu et al. 2022).

22.6.5.1.1 Pest Resistance

One of the key reasons for integrating wild relatives into sugarcane breeding programs is their potential to enhance pest resistance. Sugarcane is vulnerable to numerous pests, such as borers, aphids, and root-feeding nematodes, which can cause substantial damage to crops. Wild relatives often possess natural resistance mechanisms that are not present in cultivated sugarcane, making them valuable sources of pest-resistance traits (Miedaner and Juroszek 2021).

22.6.5.1.2 Pest Resistance Traits in Wild Relatives

- *Saccharum spontaneum*, for instance, exhibits resistance to various insect pests, mainly stem borers (*Scirpophagaexcerptalis*) and other lepidopteran pests, due to its thicker cuticle and higher levels of defensive chemicals, such as phenolic compounds(Miedaner and Juroszek 2021).
- *Erianthus arundinaceus* has been shown to possess physical defenses, such as thicker, more fibrous stems, which make it less palatable to pests(Miedaner and Juroszek 2021).

These beneficial traits can be introduced into cultivated sugarcane through traditional breeding methods or advanced biotechnological approaches, such as marker-assisted selection (MAS) or genetic modification (GM). By integrating traits from wild relatives, sugarcane cultivars can be more resistant to pests, reducing the need for chemical pesticides and supporting more sustainable agricultural practices (Miedaner and Juroszek 2021).

22.6.5.2 Improved Yield and Biomass Production

Wild sugarcane relatives can also improve yield potential and biomass production. For example, wild species like *Erianthus arundinaceus* and *Miscanthus,* are known for their high biomass yield potential. While sugarcane is primarily grown for its sugar content, there is increasing interest in using sugarcane as a bioenergy crop to produce ethanol and other biofuels (Verma et al. 2020a, b; Chen et al. 2020). Wild relatives with higher biomass potential can be crossed with cultivated sugarcane to improve yields in terms of sugar content and total biomass (Xu et al. 2022; Rodríguez et al. 2024).

22.6.5.2.1 Biomass and Biofuel Production

- *Miscanthus* and *Erianthus* species have high potential for biofuel production due to their rapid growth and biomass accumulation, which can enhance sugarcane's role in bioenergy production.
- Transferring high-biomass traits from wild relatives can help sugarcane adapt to bioenergy markets while increasing its overall economic value.

22.6.5.3 Challenges and Considerations

While the potential benefits of incorporating wild relatives into sugarcane breeding programs are significant, several challenges remain:

- *Hybrid Incompatibility*: Some wild relatives of sugarcane, particularly *S. spontaneum*, are distantly related to cultivated sugarcane and may not easily hybridize due to differences in chromosome numbers and other genetic barriers.
- *Gene Introgression*: Even when hybridization is successful, introgressing desirable traits from wild relatives into cultivated sugarcane can be a long and complex process, requiring multiple breeding generations.
- *Regulatory Hurdles*: The use of wild relatives in sugarcane breeding, particularly in genetic modification or gene-editing contexts, can face regulatory challenges, especially concerning the movement of transgenes into non-GM crops or wild populations.

22.7 Future Perspectives

Future efforts in sugarcane breeding should focus on addressing the primary challenges posed by biotic stressors. Modern genome-editing technologies, such as CRISPR/Cas9 and base editing, offer promising solutions for developing stress-tolerant and pest-resistant varieties. However, further research is necessary to

realize these possibilities fully. Integrating these advanced techniques with traditional breeding approaches will contribute to the sustainable production of sugarcane, enabling better management of environmental stresses in the face of climate change. Additionally, plant epigenetics comprising mechanisms like histone modification, DNA methylation, noncoding RNA, and chromatin remodeling, emerges as a valuable tool for gaining deeper insights into the biological processes of sugarcane. Given sugarcane's vegetative propagation, narrow genetic diversity, and complex genome, it is crucial to employ molecular breeding tools to enhance cultivars. A key step toward successful crop improvement will be fostering open communication between molecular biologists, plant physiologists, farmers, breeding companies, and the public to navigate agricultural biotechnology's economic, sociological, legal, and ethical challenges. Future efforts must focus on ensuring these new technologies' safety, ecological stability, and transparency. Education and outreach efforts to inform the public about the benefits and safety of genetically modified sugarcane will be essential to garnering support for these approaches.

22.8 Conclusion

Sugarcane, a critical industrial cash crop globally, has undergone continuous improvement in agronomic traits and resistance to both biotic and abiotic stress since ancient times. Stem borers significantly threaten sugarcane productivity, causing direct physical damage and indirectly increasing susceptibility to diseases. By understanding natural resistance mechanisms, such as physical barriers, chemical defenses, and behavioral responses, researchers can identify and breed for traits that enhance resistance. However, conventional breeding methods are often time-consuming and insufficiently efficient to produce the improved sugarcane varieties required in the face of ongoing climate change. In contrast, advances in plant genetic manipulation using molecular tools have proven highly beneficial for achieving sustainable, high-yield crops with reduced susceptibility to biotic stresses. Technologies like molecular marker-assisted breeding, genetic transformation, and genome editing techniques, such as CRISPR, hold great potential for accelerating sugarcane genetic improvement.

Marker-assisted breeding, in particular, has been used to pyramid multiple stress-tolerant genes or quantitative trait loci (QTLs), helping to develop robust sugarcane varieties better suited to cope with environmental challenges and ensure food security. Modern biotechnological approaches promise to deliver genetically enhanced cultivars with improved physiological, morphological, and agronomic traits, offering superior field performance under varying environmental conditions and in a fraction of the time required for traditional breeding. As breeders continue to make progress in identifying and incorporating resistance traits, sugarcane cultivation will become more sustainable, productive, and environmentally friendly. However, overcoming the challenges of hybridization barriers, regulatory approval for transgenic crops, and public acceptance will be keys to realizing the full potential of these innovative breeding strategies.

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Chapter 23
Genetic Improvement for Herbicide Tolerance in Sugarcane

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Abstract Sugarcane (*Saccharum officinarum*) is a critical global crop, primarily cultivated for sugar and ethanol production, and its effective management is vital for optimal yields. Weeds are a significant biotic stressor in sugarcane farming, competing for resources and causing substantial yield losses. While traditional hand weeding has been largely replaced by mechanical control, the high-energy demands and associated environmental concerns of mechanical methods have led to the widespread adoption of chemical herbicides. Long-term reliance on broad-spectrum herbicide weed control has led to resistance evolution in weeds in sugarcane fields. Given these challenges, breeding herbicide-tolerant sugarcane varieties is becoming increasingly important. Sugarcane's polyploid nature, long breeding cycles, and limited genetic diversity pose significant barriers to improving herbicide tolerance through conventional breeding. However, advancements in genetic engineering and gene editing techniques, such as CRISPR-Cas9, promise to enhance herbicide resistance in sugarcane. This chapter reviews the current state of herbicide tolerance breeding in sugarcane, discussing biochemical mechanisms of herbicide action, conventional and molecular breeding strategies, and the potential of gene editing to

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develop herbicide-tolerant varieties. Understanding these methodologies is essential for navigating the future of herbicide use in sugarcane farming and achieving sustainable, high-yield production in the face of evolving agricultural challenges.

Keywords Sugarcane · Weed competition · Herbicide-tolerance · Molecular breeding · CRISPR-Cas9

23.1 Introduction

Sugarcane (*Saccharum officinarum* L.) is an important crop around the world. It is mainly used for producing sugar and ethanol and plays a key role in the farming economies of various tropical and subtropical regions (Budeguer et al. 2021; Verma et al. 2019a, b; Chen et al. 2024). As a high-input crop, its cultivation faces several challenges, particularly from weeds that compete with sugarcane for nutrients, water, and sunlight (Verma et al. 2020a, b, 2021). Effective weed management is critical to achieving optimal yields, and herbicide application has become a cornerstone of modern sugarcane farming (Budeguer et al. 2021; Afghan et al. 2024). Gharde et al. (2018) found that weeds cause huge losses in India, estimated at 11 billion USD each year. These losses vary by crop, from 13.8% in transplanted rice to as high as 76% in soybeans. Among all living factors that harm crops, weeds cause the most damage (34%), while insects (18%) and diseases (16%) have a smaller impact (Schulz and Segobye 2016; Li et al. 2023). Therefore, controlling weeds properly is necessary in any crop production system to achieve high yields and good quality (Budeguer et al. 2021).

Hand weeding was once the main method of controlling weeds, but it was slowly replaced by mechanical control (Ofosu et al. 2023). Mechanical weed control practices are now considered unsatisfactory due to the high-energy requirements, associated costs, and the perceived facilitation of soil erosion and compaction (Liu et al. 2021; Verma et al. 2024a). Therefore, it has largely been replaced by chemical weed control, as it removes weeds from crops with minimal soil disturbance (Travlos et al. 2020). Herbicide usage worldwide has increased 15-fold after the introduction of transgenic herbicide tolerance cultivars, which offer the opportunity to control weeds through need-based applications of herbicides (Ofosu et al. 2023; Verma et al. 2024b). Herbicides have strong effects on plant growth and health, such as dead spots, late flowering, misshaped flowers, slow growth, curled leaves, and burn symptoms, etc. A good herbicide should effectively kill weeds but should not stay in the environment for a long time (Travlos et al. 2020).

Most broad-spectrum herbicides are not selective, which limits their use in certain farming practices. However, they are very useful for complete weed control. Broad-spectrum herbicides are limited because of their crop sensitivity (Ofosu et al. 2023). Repeated use of selective herbicides has led to weeds developing herbicide

resistance (Ofosu et al. 2023). Therefore, from the current perspective, there is a need for a genotype with herbicide tolerance. However, the genetic enhancement of herbicide tolerance in sugarcane has challenges (Ofosu et al. 2023). Sugarcane is a complex, polyploid crop with a long breeding cycle, limited genetic diversity, and difficulties in genetic transformation (Liang et al. 2024; Li et al. 2024; Ali et al. 2024).

Nonetheless, significant progress has been made in understanding the molecular mechanisms behind herbicide resistance and identifying potential genetic targets (Mucheri et al. 2024). Transgenic sugarcane varieties have been developed, displaying resistance to specific herbicides, and recent advances in gene editing technologies, such as CRISPR-Cas9, offer new avenues for precise and efficient genetic modifications (Surya Krishna et al. 2023; Verma et al. 2023; Huang et al. 2024). The development of herbicide-tolerant crops is becoming more important due to the increasing cost of hand weeding, a shortage of farm workers, reduced herbicide prices, better effectiveness of new herbicides, and the rising need for mechanization in intensive farming (Ali et al. 2024). Breeding for any crop trait requires variation in that trait, and if variation is not present, it must be created and then used to improve the crop (Song et al. 2019; Ofosu et al. 2023). Crop genotype tolerant to herbicide endows us with an opportunity to have mechanized agriculture (Nosratti and Chauhan 2023).

Herbicide tolerance in sugarcane refers to the ability of a sugarcane variety to withstand the effects of herbicides, allowing it to survive and grow even when exposed to a specific herbicide application, often achieved through genetic modification or by selecting naturally occurring tolerant sugarcane genotypes; this is particularly important for weed control in sugarcane fields, enabling farmers to selectively eliminate weeds without harming the crop.

Therefore, in this chapter, we have elaborated on the recent methodologies applied for herbicide tolerance crop breeding, biochemical mechanisms of herbicide action in sugarcane, conventional breeding methods for selecting herbicide-tolerant sugarcane, genetic engineering in sugarcane, gene editing, and future course of action. By understanding the potential and limitations of these approaches, we can better assess the role of herbicide-tolerant sugarcane in the evolving landscape of global agriculture.

23.2 Biochemical Mechanisms of Herbicide Action in Sugarcane

Herbicides are chemicals designed to control or eliminate unwanted plant species, such as weeds, by interfering with their biochemical and physiological processes (Nath et al. 2024). The general mechanism of herbicide action via pre-emergence / post-emergence of weeds are illustrated in Fig. 23.1.

In sugarcane, as in other crops, herbicides primarily exert their effects by inhibiting essential metabolic processes, disrupting cellular functions, or causing

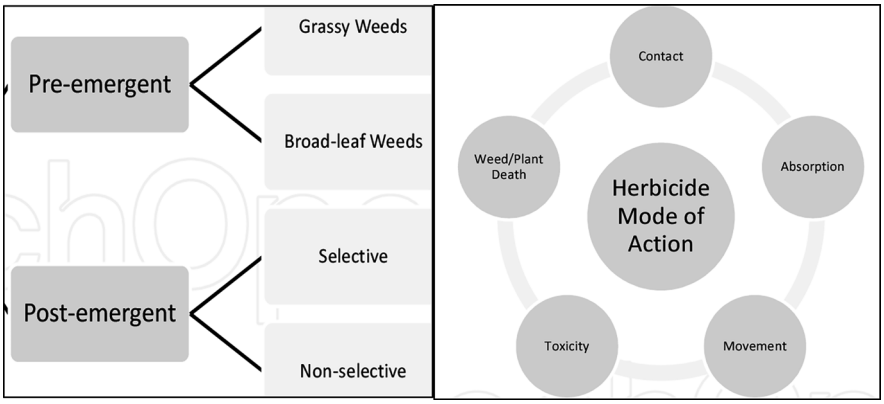

Fig. 23.1 The general mechanisms of herbicide action in weeds

irreversible damage to key enzymes and proteins (Mucheri et al. 2024). The mechanisms of herbicide action are described in the following heads.

23.2.1 Inhibition of Photosynthesis

Many herbicides target photosynthesis, the critical process by which plants convert light energy into chemical energy to produce sugars (Khan et al. 2024). Herbicides that inhibit photosynthesis typically interfere with the light reactions or electron transport chain in chloroplasts (Twitty and Dayan 2024). A well-known example is atrazine, which belongs to the triazine class of herbicides (Khan et al. 2024). Atrazine inhibits the enzyme photosystem II (PSII), a component of the photosynthetic electron transport chain (Nishikawa et al. 2024). This leads to the accumulation of toxic reactive oxygen species (ROS) in plant cells, damaging cell membranes and resulting in the plant's eventual death (Sharma et al. 2021, 2022; Khan et al. 2024). In sugarcane, herbicide tolerance to this class of chemicals is typically achieved by altering the target site or enhancing detoxification mechanisms.

23.2.2 Disruption of Amino Acid Synthesis

Some herbicides prevent the production of essential amino acids, which are the building blocks for proteins and other cell components (Cai et al. 2023). For example, glyphosate is a widely used herbicide that targets the enzyme 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) (Xu et al. 2024). EPSPS is a key enzyme in the shikimate pathway, responsible for synthesizing aromatic amino acids, such as phenylalanine, tryptophan, and tyrosine (Xu et al. 2024). Glyphosate binds to

EPSPS, preventing the formation of these amino acids, ultimately disrupts protein synthesis and leading to plant demise (Faizal et al. 2024). Herbicide-tolerant sugarcane varieties can be developed by introducing a glyphosate-resistant EPSPS gene from a nonplant organism, such as *Agrobacterium*, or by increasing the activity of detoxifying enzymes to metabolize glyphosate (Ofosu et al. 2023).

23.2.3 Inhibition of Lipid Synthesis

Lipids are vital components of cell membranes and are involved in various cellular processes such as energy storage and signaling (Liang et al. 2023). Herbicides that disrupt lipid synthesis, such as saflufenacil, target enzymes involved in fatty acid biosynthesis (Odero et al. 2023). These herbicides block acetyl-CoA carboxylase (ACCase), an enzyme that produces long-chain fatty acids crucial for membrane integrity and cellular function (Blyth et al. 2024). When lipid biosynthesis is inhibited, plant cells lose their structural integrity, leading to cell death (Liang et al. 2023). Sugarcane tolerance to these herbicides can be engineered by modifying the expression or structure of ACCase, thus rendering the herbicide less effective (Liang et al. 2023).

23.2.4 Disruption of Microtubule Function

Herbicides that interfere with the cytoskeleton, particularly microtubules, can cause disruptions in cell division and growth (Duke et al. 2023). Dinitroaniline herbicides (e.g., oryzalin and pendimethalin) are known to inhibit microtubule formation by binding to tubulin, a protein that forms microtubules (Duke et al. 2023). Microtubules are essential for cell division, intracellular transport, and overall plant growth (Duke et al. 2023). When these herbicides disrupt microtubule formation, cell division is impaired, leading to plant growth inhibition and, eventually, plant death (Duke et al. 2023). Sugarcane plants with tolerance to dinitroaniline herbicides may possess mutations in tubulin genes or mechanisms that sequester or degrade the herbicide before it can interfere with microtubule assembly (Ali and Khan 2023).

23.2.5 Target-Site Resistance Mechanisms

Target-site resistance (TSR) arises when an herbicide's target enzyme or protein is altered so that the herbicide can no longer bind to or prevent its target effectively (Kumar et al. 2023). This type of resistance is typically associated with genetic mutations that modify the structure of the herbicide's target site, reducing the herbicide's ability to disrupt essential plant processes (Ofosu et al. 2023). These genetic

mutations can reduce the affinity of the herbicide for its target, making the herbicide less effective or rendering it ineffective altogether (Kumar et al. 2023). For instance, target-site mutations in the EPSPS enzyme can confer resistance to glyphosate, as these mutations reduce the herbicide's ability to bind to the enzyme (Kumar et al. 2023). Similarly, mutations in enzymes such as acetohydroxyacid synthase (AHAS), the target of imidazolinone and sulfonylurea herbicides, can make sugarcane plants less susceptible to these herbicides (Kumar et al. 2023).

A small change in a single nucleotide of the gene that makes a protein targeted by a herbicide can lead to a single amino acid change. This can stop the herbicide from binding to the protein while still allowing the enzyme to function properly (Gwatidzo et al. 2023). In most target-site proteins, only a few amino acids in or near the herbicide-binding site can be replaced in a way that leads to TSR (Pamirsky et al. 2023). Most target-site mutations occur in or near the herbicide binding site, but some mutations arise elsewhere in the protein structure (Pamirsky et al. 2023). Target-site mutations are identified based on the amino acid and its position in the protein, starting from the first codon (Gwatidzo et al. 2023). In some cases, these mutations provide strong resistance, while in others, they offer lower but still significant resistance (Gwatidzo et al. 2023). Some target-site mutations weaken the enzyme's normal function, while others allow it to function almost completely. Besides single nucleotide changes, the deletion of an entire codon can also reduce the ability of herbicides to bind to the target enzyme (Gwatidzo et al. 2023). It is important to note that the same molecular process, such as a single nucleotide change causing an amino acid substitution, plays a key role in resistance across various herbicide action sites (Kumar et al. 2023).

TSR mechanisms are specialized and apply to only one site of action. Whether a specific target-site mutation that provides resistance to one herbicide also gives resistance to other chemicals in the same site-of-action group depends on how each herbicide interacts with the target protein (Kumar et al. 2023). TSR can also be due to increased expression of the target-site gene, producing more enzymes than can be substantially inhibited by typical herbicide application rates (Mucheri et al. 2024). Increased gene expression can be due to regulatory changes increasing transcription and/or increased genomic copy number of the target-site gene, also resulting in increased transcription (Muthu Lakshmi Bavithra et al. 2023). Nonsynonymous SNPs imparting resistance to a herbicide target site is the most common mechanism of TSR (Mucheri et al. 2024). These mutations may be within or in the proximity of the catalytic domain of an enzyme and affect a herbicide's ability to compete for the binding of a substrate, or these mutations may affect other domains of enzymes and proteins (Mucheri et al. 2024).

23.2.5.1 Mutations Affecting Herbicide Binding

The first discovered target-site mutation was for the photosystem II (PSII)-inhibiting herbicides, which compete with plastoquinone for binding on the D1 protein encoded by the psbA gene and thereby inhibit PSII electron transport (Ali and Khan

2023). Amino acid substitutions in the psbA gene typically confer high-level resistance to a single chemical family but not to herbicides from other families or groups (Kumar et al. 2023). For example, the single amino acid change S264G confers high-level resistance to the triazine herbicides in numerous species around the world but moderate to no resistance to the other families of PSII inhibitors, including the triazinones, which are in the same group as the triazines (Kumar et al. 2023). The substitution of Gly for Ser at position 264 prevents triazine binding but also compromises plastoquinone binding and impairs photosynthesis, resulting in a strong fitness penalty (Gwatidzo et al. 2023).

23.2.6 Non-target-Site Resistance (Enhanced Herbicide Metabolism)

Non-target-site resistance (NTSR) involves resistance mechanisms that do not directly alter the herbicide's target site but instead work to reduce the herbicide's overall impact on the plant (Cai et al. 2023). These mechanisms can prevent the herbicide from entering the plant, reduce its translocation to sensitive tissues, or enhance its detoxification. Plants can detoxify herbicides by modifying or degrading the active chemical compounds (Ali and Khan 2023). This process is carried out by cytochrome P450 monooxygenases, glutathione S-transferases (GSTs), and carboxylesterases (Cai et al. 2023). These enzymes facilitate the breakdown or modification of herbicides into less toxic compounds, preventing them from reaching their target site in the plant (Ali and Khan 2023). Sugarcane varieties with increased expression of these detoxifying enzymes may exhibit higher levels of herbicide tolerance, allowing for effective weed control without harming the crop (Kumar et al. 2023). There are no cases of evolved resistance to soil-applied herbicides due to reduced root absorption. Early work on differential foliar absorption of herbicides between species was attributed mainly to differences in cuticle thickness and/or composition (Kumar et al. 2023). Still, the number and/or structures of leaf trichomes and hairs have also been implicated (Cai et al. 2023). Hirsute leaves are covered with hairy trichomes that can retain spray droplets better than smooth, hairless, or glandless cuticles, facilitating absorption. Other leaves have lysigenous glands that produce and store oily secondary metabolites that can compartmentalize lipophilic herbicides, preventing them from reaching their site of action (Dinesh Babu et al. 2022). Differences in foliar absorption of herbicides between plants have been attributed to leaf anatomical features rather than any biochemical differences (Dinesh Babu et al. 2022).

23.2.6.1 Reduced Herbicide Uptake and Translocation

One of the key non-target-site resistance mechanisms is reduced herbicide uptake (Mucheri et al. 2024). This can occur through changes in the plant's cell membrane properties or the activity of transport proteins that regulate the movement of substances across cell membranes (Mucheri et al. 2024). For example, changes in the cuticular wax *or* cell wall composition may reduce the absorption of herbicides, preventing them from entering the plant and reaching their target sites (Mucheri et al. 2024). Similarly, reduced translocation of herbicides within the plant can also contribute to tolerance. Herbicides that generally move from the application site to other parts of the plant are less able to spread, reducing their toxicity in non-target tissues (Butova et al. 2024).

23.2.6.2 Enhanced Sequestration

Once herbicides enter the plant, they must often be transported to specific cellular compartments, such as the vacuole, to prevent toxic effects. Enhanced sequestration refers to the plant's ability to sequester herbicides into vacuoles or other organelles, effectively isolating them from critical biochemical pathways (Butova et al. 2024). This can limit the herbicide's ability to affect cellular processes, such as protein synthesis or cell division, which are essential for plant growth. Herbicide tolerance can also arise through changes in how the plant absorbs, translocates, or stores herbicides. Reduced herbicide uptake can limit the amount of active herbicide reaching sensitive tissues, while enhanced sequestration involves compartmentalizing the herbicide in vacuoles, preventing its harmful effects on cellular machinery (Butova et al. 2024). These mechanisms help mitigate the adverse effects of herbicides, ensuring that the herbicide either does not reach its target site or is rendered inactive (Butova et al. 2024).

23.2.6.3 Herbicide Detoxification (Metabolic Resistance)

Many herbicides can be detoxified by plants through the action of specialized enzymes that modify the herbicide, rendering it less toxic (Shahid et al. 2023). Non-target-site metabolic resistance involves the upregulation of detoxifying enzymes, such as cytochrome P450 monooxygenases, glutathione S-transferases (GSTs), and carboxylesterases, which degrade or modify herbicides, reducing their toxic effects (Shahid et al. 2023). In sugarcane, these enzymes could break down herbicides, such as glyphosate, glufosinate, or paraquat, preventing them from accumulating to harmful levels (Shahid et al. 2023).

- Cytochrome P450s are involved in oxidative metabolism, where they add oxygen atoms to herbicides, making them more water-soluble and more accessible for the plant to excrete (Das et al. 2023).

- Glutathione S-transferases (GSTs) facilitate the conjugation of herbicides with glutathione, a tripeptide that neutralizes the herbicide's toxic effects and helps remove it from the plant (Das et al. 2023).
- Carboxylesterases hydrolyze ester bonds in herbicides, rendering them less toxic and promoting their elimination (Das et al. 2023).

23.2.6.4 Disruption of Protein Synthesis

Some herbicides, such as glufosinate-ammonium, inhibit the enzyme glutamine synthetase. This enzyme is crucial for nitrogen assimilation, as it converts ammonia into glutamine, an essential nitrogen-containing amino acid (Takano and Dayan 2020). Inhibition of glutamine synthetase by glufosinate leads to the accumulation of toxic ammonia in plant cells, causing cellular damage and plant death. Herbicide tolerance in sugarcane varieties to such chemicals could be achieved by overexpressing the glutamine synthetase enzyme or introducing modified versions less sensitive to inhibition (Takano and Dayan 2020).

23.3 Conventional Breeding Methods for Selecting Herbicide-Tolerant Sugarcane Varieties

Herbicides are a commonly used tool for controlling weeds. Still, the development of herbicide resistance in both sugarcane and weed species has raised the need for more effective weed management strategies (Suganthi et al. 2019). One promising approach to addressing this challenge is the development of herbicide-tolerant sugarcane varieties, enabling farmers to use herbicides more effectively and reduce their environmental impact (Dinesh Babu et al. 2022).The first attempt to introduce herbicide resistance in sugarcane was made by Chowdhury and Vasil (1992) when they transformed suspension culture cells by microprojectile bombardment and by electroporation of protoplasts with a plasmid containing the bacterial gene bar (phosphinothricin acetyltransferase), a selectable marker which confers resistance to the herbicide (glufosinate ammonium). Colonies resistant to Basta from both transformation systems were recovered, and stable integration of the bar gene was confirmed by Southern blot analysis.

However, no whole plants were regenerated since cell lines were old and non-morphogenic. Glufosinate ammonium is a nonselective pro herbicide that plants convert into the phytotoxin phosphinothricin (PPT). The herbicide inhibits the essential ammonia assimilation enzyme glutamine synthetase (Mulwa and Mwanza 2006). The first transgenic sugarcane expressing the bar gene was produced by Gallo-Meagher and Irvine (1996) by biolistic transformation of embryogenic calli. When the field performance of glufosinate ammonium-resistant transformants was assessed, stable transgene expression was observed over several ratoons with

repeated herbicide Ignite application, either to leaf segments or to the entire plant with lethal dosages for nontransformed control plants. Interestingly, when transgenic events were propagated through meristem propagation culture, resistance was conserved, and these results demonstrated that the bar could be used as an effective selectable marker for sugarcane transformation. Likewise, Snyman et al. (1998) transformed the embryogenic callus of the same variety, NCO310, by microprojectile bombardment using a vector containing a synthetic pat gene, which confers resistance to glufosinate ammonium.

Modern biotechnological approaches, such as genetic engineering and gene editing, have received significant attention, and conventional breeding methods remain a critical strategy for selecting herbicide-tolerant sugarcane varieties (Suganthi et al. 2019). Conventional breeding involves selecting and crossbreeding parent plants with desirable traits to produce offspring with improved characteristics (Begna 2021). In the context of herbicide tolerance, conventional breeding techniques focus on incorporating natural variations in herbicide tolerance into high-yielding sugarcane cultivars. This section outlines the conventional breeding methods used to develop herbicide-tolerant sugarcane varieties, discussing strategies, such as selection, hybridization, mutation breeding, and backcrossing, as well as the challenges and limitations associated with these methods.

23.3.1 Selection of Herbicide-Tolerant Traits

The first step in conventional breeding for herbicide tolerance in sugarcane is identifying and selecting parental plants with natural or induced tolerance to specific herbicides. This can be achieved through both phenotypic selection and genotypic selection, where traits of interest are either visually assessed or identified at the genetic level (Wang et al. 2017) (Table 23.1).

23.3.1.1 Phenotypic Selection

Phenotypic selection involves the observation of plant traits in response to herbicide treatment (Wang et al. 2024). Sugarcane plants that exhibit tolerance to herbicides will show fewer visible symptoms of injury, such as leaf chlorosis, stunting, or necrosis when exposed to herbicide application (Wang et al. 2024). These tolerant plants are then selected for further breeding. Phenotypic selection requires rigorous screening of a large number of sugarcane plants under controlled conditions to ensure that the desired trait is heritable and stable across generations (Wang et al. 2024).

- Screening populations: Sugarcane breeding programs often screen large populations of sugarcane for herbicide tolerance. These populations may include

Table 23.1 Genetic modification for herbicide-tolerant traits in sugarcane

Variety	Trait method	Explants	Promotor	Gene	References
SP80-180	Particle bombardment	Axillary bud	Ubi-1	Bar	Falco et al. (2000)
ROC22	Agrobacterium mediated	Embryogenic calli	Ubi-1	EPSPS Cry1Ab	Wang et al. (2017)
CPF-234 CPF-213 CPF-246	Particle bombardment	Embryogenic calli	CaMV35S	Glyphosate	Nasir et al. (2014)
TUC 03-12	Particle bombardment	Embryogenic calli	Rice action	EPSPS	Racedo et al. (2023)
CPF-246	Agrobacterium mediated	Embryogenic calli	Ubi-1	Cry1Ac, Cry2 & GT	Qamar et al. (2021)
ROC22	Agrobacterium mediated	Embryogenic calli	CaMV35S	Bar	Zan et al. (2023)
ROC22	Agrobacterium mediated	Embryogenic calli	Ubi-1	Bar	Wang et al. (2024)
RA87-3	Particle bombardment	Embryogenic calli	Rice action	EPSPS	Noguera et al. (2019)
NCo310	Particle bombardment	Meristem		Pat	Leibbrandt and Snyman (2003)
Ja60-15	Agrobacterium mediated	Embryogenic calli	Rice-Ubi	Bar	Noguera et al. (2019)
Co92061 and Co671	Agrobacterium mediated	Somatic embryos	CaMV35S	Bar	Manickavasagam et al. (2004)

existing varieties, wild relatives, or progeny from previous hybridizations (Racedo et al. 2023).

- Herbicide dose-response tests: In these tests, plants are exposed to varying herbicide concentrations to assess their tolerance. Plants that exhibit lower levels of damage at higher herbicide concentrations are considered more tolerant and may be selected for further breeding (Racedo et al. 2023).

23.3.1.2 Genotypic Selection

Genotypic selection involves identifying the genetic basis of herbicide tolerance. Molecular biology and genomics advances have identified specific genes or genetic markers associated with herbicide tolerance (Hasan et al. 2021). Marker-assisted selection (MAS) is an increasingly popular method that allows breeders to select plants with desirable traits at the genetic level without relying solely on phenotypic expression (Hasan et al. 2021).

- Identification of molecular markers: Researchers can identify molecular markers linked to herbicide tolerance genes using genetic mapping techniques or genomic tools, such as genome-wide association studies (GWAS). These markers can then

be used in breeding programs to accelerate the selection of herbicide-tolerant plants (Racedo et al. 2023).
- Genetic analysis of parents: By analyzing the genetic profiles of potential parental lines, breeders can choose the most favorable combinations of genes for herbicide tolerance. This approach reduces the time and resources required to identify tolerant plants by phenotype alone (Hasan et al. 2021).

23.3.2 Hybridization for Herbicide Tolerance

Hybridization is a common breeding method used to combine desirable traits from different parent plants into a single offspring (Wang et al. 2017). In the case of herbicide tolerance, breeders cross sugarcane varieties with known tolerance to herbicides with high-yielding varieties or those with desirable agronomic characteristics (Wang et al. 2017).

23.3.2.1 Crossbreeding Technology

Crossbreeding is mating two genetically distinct sugarcane plants to produce hybrids with improved traits (Cursi et al. 2022). When developing herbicide-tolerant sugarcane, breeders typically cross a tolerant variety with a high-performing variety that may not necessarily possess herbicide tolerance. The offspring of these crosses are then evaluated for herbicide tolerance and agronomic traits such as yield, disease resistance, and environmental adaptability (Cursi et al. 2022).

- F1 hybrid generation: The first generation (F1) of the cross is evaluated for herbicide tolerance. If the F1 hybrids show promise, they can be self-pollinated or backcrossed to stabilize the trait in subsequent generations (Cursi et al. 2022).
- Recurrent selection: Recurrent selection involves the repeated crossing of selected plants over several generations to increase the frequency of the herbicide tolerance trait in the population while maintaining desirable yield and quality traits (Cursi et al. 2022).

23.3.2.2 Backcrossing

Backcrossing is a technique where a hybrid plant is crossed back to one of its parent plants (typically the herbicide-tolerant parent) to reinforce the herbicide-tolerance trait (Vats 2015). Backcrossing helps to introduce specific traits from one parent into a population while minimizing the introduction of undesirable characteristics from the other parent (Vats 2015).

- Backcrossing with elite varieties: In sugarcane, backcrossing can introduce herbicide tolerance into elite sugarcane varieties that already have desirable

agronomic traits. The progeny becomes more similar to the elite variety through successive backcrosses but with the added herbicide tolerance trait (Vats 2015).
- Gene introgression: This process involves gradually introducing herbicide tolerance genes into a high-yielding variety through backcrossing, ensuring that the resulting plants retain the desirable agronomic traits while acquiring tolerance (Vats 2015).

23.3.3 Mutation Breeding

Mutation breeding is a conventional technique that involves using physical or chemical mutagens to induce plant genetic mutations. These mutations may lead to the development of herbicide tolerance (Ali et al. 2024). While mutation breeding is less targeted than genetic engineering, it can effectively create variation in traits such as herbicide tolerance (Ali et al. 2024).

23.3.3.1 Induced Mutagenesis

Sugarcane breeders may treat sugarcane seeds or vegetative material with physical mutagens (e.g., radiation) or chemical mutagens (e.g., ethyl methanesulfonate) to induce genetic mutations (Nosratti and Chauhan 2023). These mutations may affect the plant's response to herbicides by altering the target site of herbicide action or enhancing detoxification mechanisms. After mutagenesis, the plants are grown, and those that exhibit herbicide tolerance is selected for further breeding (Nosratti and Chauhan 2023).

- M2 generation screening: In mutation breeding, the M2 generation (second generation after mutagenesis) is typically screened for herbicide tolerance. Plants that survive herbicide treatment are evaluated for their stability and heritability of the trait (Nosratti and Chauhan 2023).
- Mutation hotspots: Some studies use mutation breeding to target specific biochemical pathways involved in herbicide resistance, such as those responsible for metabolic detoxification or target-site modification (Nosratti and Chauhan 2023).

23.3.4 Challenges and Limitations of Conventional Breeding for Herbicide Tolerance

While conventional breeding methods have been effective in developing herbicide-tolerant sugarcane, there are several challenges and limitations associated with these approaches:

- Complexity of the sugarcane genome: Sugarcane is a polyploid crop with a large and complex genome, making it difficult to achieve precise genetic improvements. The large genome size and multiple gene copies complicate the identification and introgression of herbicide tolerance traits (Racedo et al. 2023).
- Long breeding cycle: Sugarcane has a long breeding cycle, typically requiring several years to produce new varieties with stable traits. This slow pace can limit the ability to respond rapidly to evolving herbicide resistance in weed populations (Racedo et al. 2023).
- Limited genetic diversity: Sugarcane breeding relies on a relatively narrow genetic base, which can constrain the diversity of herbicide tolerance traits available for selection. The introgression of traits from wild relatives or other *Saccharum* species may help address this limitation, but it requires careful management to avoid the introduction of undesirable traits (Racedo et al. 2023).
- Herbicide resistance management: Over-reliance on herbicide-tolerant varieties can contribute to developing herbicide-resistant weed populations. To manage this risk, herbicide-tolerant sugarcane should be integrated into comprehensive weed management systems, including crop rotation, mechanical weed control, and other agronomic practices (Racedo et al. 2023).

23.4 Overview of Genetic Engineering in Sugarcane

Genetic engineering involves directly manipulating an organism's genome using biotechnology tools, such as recombinant DNA technology, to introduce or modify new genes (Nosratti and Chauhan 2023). Unlike conventional breeding, which relies on natural genetic variation and selection, genetic engineering enables the precise insertion or modification of specific genes to achieve desired traits in the target organism (Nosratti and Chauhan 2023). In sugarcane, genetic engineering offers a faster and more targeted approach to improving desirable characteristics, including herbicide tolerance, pest resistance, disease resistance, and stress tolerance.

Steps Involved in the Genetic Engineering of Sugarcane

- Gene Identification and Isolation: The first step in genetic engineering is identifying the gene or genes responsible for the desired trait (e.g., herbicide tolerance). Genes may be isolated from other plants, microbes, or even synthetic sources (Mucheri et al. 2024).
- Gene Cloning and Modification: If necessary, the gene of interest is cloned and modified to ensure that it functions effectively in sugarcane cells. This may involve optimizing the gene for sugarcane expression, such as adding plant-specific promoters or signal sequences (Surya Krishna et al. 2023).
- Gene Delivery: The modified gene is introduced into sugarcane cells using one of several transformation methods. The most common methods for introducing foreign genes into sugarcane cells include:

- *Agrobacterium*-mediated transformation: This is the most widely used method, where the bacterium *Agrobacterium tumefaciens* transfers the desired gene into the plant genome (Surya Krishna et al. 2023).
- Biolistics (Gene Gun): This method involves shooting microscopic gold or tungsten particles coated with DNA into sugarcane cells. The DNA may then integrate into the plant's genome (Surya Krishna et al. 2023).
- Regeneration of Transgenic Plants: After the gene has been incorporated into the sugarcane genome, the transformed cells are cultured to regenerate whole plants. This step typically involves tissue culture techniques to grow plants from single cells or small tissue samples (Cursi et al. 2022).
- Selection and Screening: Transgenic plants are selected based on their ability to express the desired trait, often using a selectable marker (e.g., resistance to an antibiotic or herbicide). The plants are then tested for stability and heritability of the trait (Cursi et al. 2022).
- Field Trials and Commercialization: After extensive laboratory and greenhouse evaluations, transgenic sugarcane plants are tested in field trials to assess their performance under natural growing conditions. If successful, they may be commercially released for large-scale cultivation (Cursi et al. 2022).

23.5 Herbicide-Tolerant Transgenic Sugarcane

One of the most prominent applications of genetic engineering in sugarcane is the development of herbicide-tolerant varieties. Herbicide resistance in weeds has become a major issue in agriculture, and herbicide-tolerant crops can be essential in managing weeds effectively while minimizing environmental impact (Nasir et al. 2014). Genetic engineering allows sugarcane to be modified to tolerate specific herbicides, enabling farmers to use these chemicals to control weeds without harming the crop.

23.5.1 Glyphosate-Tolerant Sugarcane

Glyphosate, a broad-spectrum herbicide that inhibits the enzyme 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS), has been widely used in agriculture. Glyphosate works by blocking the shikimate pathway, essential for synthesizing aromatic amino acids in plants. However, some sugarcane varieties are sensitive to glyphosate, limiting its use in weed control (Racedo et al. 2023).

23.5.2 Genetic Modification

To confer glyphosate tolerance in sugarcane typically involves the introduction of a gene encoding a glyphosate-tolerant version of EPSPS, often derived from bacteria such as *Agrobacterium* sp. or *Enterobacter* sp. These bacterial genes are modified to work efficiently in plant systems, allowing the sugarcane to continue synthesizing aromatic amino acids even in the presence of glyphosate (Racedo et al. 2023).

23.5.3 Mechanism of Tolerance

Glyphosate-tolerant sugarcane can survive herbicide application by expressing a modified EPSPS enzyme that is less sensitive to glyphosate. This allows the plant to maintain its growth and metabolic processes, even when exposed to the herbicide (Racedo et al. 2023).

23.5.4 Other Herbicide-Tolerant Genes

In addition to glyphosate tolerance, sugarcane has also been engineered to resist other herbicides, such as glufosinate and acetolactate synthase (ALS)-inhibiting herbicides. These herbicides target specific enzymes involved in plant growth, and engineering sugarcane to resist these compounds involves introducing genes that either alter the target enzyme or enhance the plant's detoxification processes (Leibbrandt and Snyman 2001).

- Glufosinate Resistance: Glufosinate works by inhibiting the enzyme glutamine synthetase, which plays a critical role in nitrogen metabolism. Transgenic sugarcane expressing a modified glutamine synthetase enzyme resistant to glufosinate can continue metabolizing nitrogen efficiently, even in the presence of the herbicide (Leibbrandt and Snyman 2001).
- ALS Inhibitor Resistance: Resistance to ALS inhibitors can be achieved by introducing mutations into the ALS enzyme or by overexpressing the enzyme, allowing the plant to bypass the inhibitory effects of the herbicide (Leibbrandt and Snyman 2001).

23.6 CRISPR-Cas9 Gene Editing: Targeted Genome Editing to Enhance Herbicide Resistance in Sugarcane

Developing herbicide-resistant sugarcane varieties could help improve weed management and ensure sustainable crop production (Oz et al. 2021). In this context, CRISPR-Cas9 gene editing represents a groundbreaking approach to modifying the sugarcane genome precisely, offering new opportunities to enhance herbicide resistance with greater accuracy and efficiency than traditional genetic modification techniques (Oz et al. 2021).

This section discusses the application of CRISPR-Cas9 gene editing technology to enhance herbicide resistance in sugarcane, highlighting the principles of CRISPR-Cas9, its potential applications, and how it can be used to develop herbicide-resistant sugarcane varieties (Oz et al. 2021).

23.6.1 Understanding CRISPR-Cas9 Gene Editing Technology

CRISPR-Cas9 is a revolutionary genome-editing technology allowing highly targeted modifications to an organism's DNA (Bhatt et al. 2022). It was originally discovered as a natural defense mechanism in bacteria, protecting against viruses by recognizing and cutting foreign genetic materials. This system has since been adapted for various organisms, including plants, animals, and humans (Bhatt et al. 2022).

The CRISPR-Cas9 system consists of two main components:

- CRISPR: A sequence of short, repetitive DNA segments in the genome, which is used to guide the Cas9 protein to a specific location in the genome (Bhatt et al. 2022).
- Cas9 protein: An endonuclease that acts as molecular scissors, making a precise cut in the DNA at the targeted location (Bhatt et al. 2022).

Once the DNA is cut, the plant's natural repair mechanisms take over by introducing a knockout (gene disruption) or inserting a new genetic sequence if provided (Bhatt et al. 2022). This allows researchers to make precise changes to the genome by disabling genes or adding new traits much more efficiently and cost-effectively than older genetic engineering techniques (Bhatt et al. 2022).

23.6.2 Potential Applications of CRISPR-Cas9 in Enhancing Herbicide Resistance in Sugarcane

Herbicide resistance in sugarcane can be achieved through several strategies, which involve modifying specific genes involved in herbicide metabolism, detoxification, or herbicide-target site interactions. CRISPR-Cas9 technology enables precise editing of these genes, offering a more direct approach to developing herbicide-tolerant varieties than conventional breeding or transgenic methods (Oz et al. 2021).

23.6.2.1 Modifying Target-Site Resistance Genes

One of the primary mechanisms of herbicide resistance is target-site resistance, where mutations in the herbicide's target enzyme or protein reduce the herbicide's effectiveness. Glyphosate, for example, targets the EPSPS (5-enolpyruvylshikimate-3-phosphate synthase) enzyme in the shikimate pathway, which is essential for plant growth. Mutations in the EPSPS gene that reduce its affinity for glyphosate have been shown to confer resistance in many plants (Oz et al. 2021).

- EPSPS modification with CRISPR-Cas9: Using CRISPR-Cas9, researchers can introduce specific mutations into the sugarcane EPSPS gene to create less sensitive glyphosate variants. This modification can help develop glyphosate-tolerant sugarcane varieties without relying on external gene sources, as in traditional transgenic approaches. The precise control offered by CRISPR-Cas9 minimizes off-target effects, an advantage over conventional mutagenesis or random breeding methods (Oz et al. 2021).

23.6.2.2 Enhancing Herbicide Detoxification Pathways

Another strategy to improve herbicide resistance is to enhance the plant's ability to detoxify or degrade herbicides, preventing them from accumulating to toxic levels in the plant. Many herbicides are broken down by enzymes such as cytochrome P450 monooxygenases, glutathione S-transferases (GSTs), and carboxylesterases, which are involved in the plant's metabolic pathways.

- Gene editing of detoxification enzymes: CRISPR-Cas9 can be used to upregulate the expression of detoxification genes, enhancing the plant's ability to metabolize and eliminate herbicides more efficiently. For instance, editing the genes encoding cytochrome P450s or GSTs could increase the plant's capacity to detoxify herbicides such as glyphosate, glufosinate, or paraquat, making sugarcane more resilient to these chemicals (Bhatt et al. 2022).

- Inducible detoxification systems: Another potential application is the creation of inducible systems where the detoxification enzymes are activated only in the presence of herbicides, reducing unnecessary energy expenditure in the absence of herbicide stress. This can be achieved by editing regulatory regions of detoxification genes or using CRISPR to target transcription factors involved in stress responses (Bhatt et al. 2022).

23.6.2.3 Modifying Herbicide Transport and Sequestration Mechanisms

Another approach to herbicide resistance involves modifying the plant's ability to limit herbicide uptake or to sequester herbicides into vacuoles or other compartments where they are less likely to cause damage. CRISPR-Cas9 can edit genes responsible for the transport and sequestration of herbicides within the plant (Han and Kim 2019).

- Enhanced sequestration: Editing the genes that regulate vacuolar transporters, such as ATP-binding cassette (ABC) transporters or tonoplast-localized transporters, can help sequester herbicides in vacuoles or other nonsensitive areas, reducing their toxic effects on the plant. By enhancing these mechanisms, sugarcane can be more tolerant to herbicides that might inhibit growth (Han and Kim 2019).
- Reduced herbicide uptake: CRISPR-Cas9 could also be used to alter membrane transport proteins involved in the uptake of herbicides, making it more difficult for the herbicide to enter the plant in the first place. By reducing herbicide absorption, sugarcane can better withstand treatments, making weed control more effective without harming the crop (Han and Kim 2019).

23.6.3 Advantages of Using CRISPR-Cas9 for Herbicide Resistance in Sugarcane

- Precision and Targeting: CRISPR-Cas9 enables highly targeted modifications at specific loci in the genome, allowing for the precise introduction of herbicide-resistance traits without the risk of random mutations associated with traditional mutagenesis or crossbreeding (Mohan et al. 2022)
- Efficiency: CRISPR-Cas9 is much faster than conventional breeding methods, significantly reducing the time required to develop herbicide-resistant sugarcane varieties. This is especially beneficial when rapid adaptation is needed to address emerging herbicide resistance issues in weeds (Han and Kim 2019).
- No Foreign DNA: One of the key benefits of CRISPR-Cas9 is that it does not necessarily require the introduction of foreign DNA (as is the case with transgenic approaches). This is important for regulatory approval and public accep-

tance, as gene-edited plants can be perceived as more "natural" compared to genetically modified organisms (GMOs) (Mohan et al. 2022)
- Potential for Multiplexing: CRISPR-Cas9 allows for the simultaneous modification of multiple genes, or multiplexing, which could enable sugarcane plants to resist numerous herbicides or exhibit enhanced resistance to a range of herbicides in a single variety. This can offer a more sustainable solution for managing weed resistance over the long term (Mohan et al. 2022)

23.6.4 Challenges and Considerations in Using CRISPR-Cas9 for Herbicide Resistance

While CRISPR-Cas9 holds great promise, there are several challenges and considerations to address before it can be widely applied in sugarcane for herbicide resistance:

- Off-Target Effects: Although CRISPR-Cas9 is highly precise, off-target effects, such as unintended modifications to other parts of the genome, are still possible. Advances in CRISPR design and delivery methods are continually being made to reduce these risks, but careful testing is needed to ensure that the edited plants are safe for commercial use (Bhatt et al. 2022).
- Gene Flow and Resistance Development: One of the concerns with herbicide-tolerant crops, including those edited using CRISPR-Cas9, is the potential for spreading herbicide resistance to neighboring plants or wild relatives through gene flow. This could exacerbate weed resistance problems over time. Managing gene flow and ensuring that herbicide-resistance traits are contained within controlled environments will be critical for the responsible use of gene-edited sugarcane (Oz et al. 2021).
- Regulatory Hurdles: The regulatory landscape for gene-edited crops varies across countries, and some regions have strict rules regarding the commercialization of gene-edited plants. Regulatory agencies must assess the safety, environmental impact, and potential risks associated with CRISPR-edited herbicide-resistant sugarcane before approval for release.
- Public Perception: Public acceptance of gene-edited crops is a key factor in their adoption. Although CRISPR-Cas9 has the potential to be seen as less controversial than traditional GMOs, concerns over food safety, environmental impact, and corporate control of genetic resources still need to be addressed (Oz et al. 2021).

23.7 Genomic Selection and Marker-Assisted Breeding: Use of Molecular Markers to Select for Traits Associated with Herbicide Tolerance in Sugarcane Breeding Programs

Herbicide application is a common method of weed control, but over-reliance on herbicides has led to increased resistance in both weeds and, in some cases, the crop itself (Kumar et al. 2023). Developing herbicide-tolerant sugarcane varieties through breeding programs is a sustainable solution to mitigate these challenges, and genomic selection (GS) and marker-assisted breeding (MAB) are two advanced tools that can enhance the efficiency and precision of such breeding efforts (Sandhu et al. 2022).

These molecular breeding techniques leverage the genetic understanding of sugarcane, using molecular markers associated with herbicide tolerance traits to select plants with desirable genetic profiles (Sandhu et al. 2022). This approach significantly accelerates the breeding process by identifying and selecting the most suitable genotypes early in the breeding cycle, improving the efficiency and success rate of developing herbicide-resistant sugarcane varieties (Sandhu et al. 2022). This section focuses on using genomic selection and marker-assisted breeding to select for herbicide tolerance in sugarcane.

23.7.1 Principles of Genomic Selection and Marker-Assisted Breeding (MAB)

23.7.1.1 Genomic Selection

Genomic selection (GS) is an advanced form of MAS that involves using high-density molecular markers across the entire genome to predict the genetic potential of a plant for a particular trait (Hasan et al. 2021). Unlike traditional breeding methods, which rely on observable characteristics or phenotypic selection, GS uses genotype data (information from molecular markers) to estimate the genetic merit of individuals for traits, such as herbicide tolerance, yield, or disease resistance (Hasan et al. 2021).

- How it works:
  1. Genome-Wide Association Studies (GWAS) or genetic mapping are first used to identify genomic regions [(QTLs or quantitative trait loci)] associated with herbicide tolerance (Hasan et al. 2021).
  2. The relationship between the markers and the trait of interest is then modeled using a statistical model [e.g., BLUP or best linear unbiased prediction] (Hasan et al. 2021).

3. These models can predict the breeding values of individuals in a population based on their marker profiles, allowing for the early selection of candidates with the best genetic potential for herbicide tolerance (Hasan et al. 2021).

- Advantages:
  - Accelerates the breeding process by selecting plants at the seedling or early growth stage, long before phenotypic expression of herbicide tolerance is visible.
  - Allows for including traits that are difficult or costly to measure, such as those related to herbicide resistance, which may not be easily observable in early generations.
  - Reduces the need for extensive field trials and phenotypic screening, thereby cutting costs and time (Hasan et al. 2021).

23.7.1.2 Marker-Assisted Breeding (MAB)

Marker-assisted breeding is a more traditional form of molecular breeding where specific molecular markers linked to traits of interest are used to select individuals carrying those markers (Sandhu et al. 2022). These markers are typically related to genes that control important characteristics, such as herbicide tolerance (Hasan et al. 2021).

- How it works:
  1. Molecular markers are identified through genetic mapping or genome-wide association studies (GWAS) that are closely linked to herbicide tolerance (Sandhu et al. 2022)
  2. These markers are then used in a breeding program to track the inheritance of desirable traits (Sandhu et al. 2022)
  3. Marker-assisted selection is typically applied in early generations to select plants that carry the desirable alleles associated with herbicide resistance (Sandhu et al. 2022)

- Advantages:
  - Marker-assisted selection is more focused on specific traits and can be applied to individual loci that directly impact herbicide tolerance.
  - It is an effective tool for introgressing specific genes for herbicide resistance into a breeding population from a donor variety.

23.7.2 Use of Molecular Markers in Herbicide Tolerance Selection in Sugarcane

Herbicide tolerance in sugarcane can arise from several mechanisms, including changes in target sites (e.g., EPSPS mutation for glyphosate tolerance), detoxification enzymes, and herbicide sequestration. Molecular markers linked to these traits can help breeders' select sugarcane varieties that are more resistant to specific herbicides, leading to more efficient weed management (Sandhu et al. 2022)

23.7.2.1 Marker Identification for Herbicide Tolerance Mechanisms

The first step in using molecular markers for herbicide tolerance is identifying genomic regions (QTLs) associated with key herbicide tolerance mechanisms (Dermawan et al. 2016). Research on herbicide tolerance in sugarcane is ongoing, but several potential marker-trait associations have been explored, including:

1. EPSPS Gene (Glyphosate Tolerance):
    - Glyphosate tolerance is primarily associated with mutations in the EPSPS (5-enolpyruvylshikimate-3-phosphate synthase) gene, which targets glyphosate. Mutations at specific sites in this gene can reduce glyphosate binding, allowing the plant to continue growing in the presence of the herbicide (Dermawan et al. 2016).
    - Molecular markers for the EPSPS gene can identify plants that carry the glyphosate-tolerant allele, enabling early selection of glyphosate-tolerant sugarcane lines (Dermawan et al. 2016).
2. P450 Cytochrome Enzymes (Metabolic Tolerance):
    - Herbicide detoxification through cytochrome P450 enzymes (P450s) plays a critical role in tolerance to herbicides, such as paraquat and glyphosate. These enzymes catalyze the breakdown of herbicides into less toxic forms (Dermawan et al. 2016).
    - Molecular markers linked to P450 genes can be used to select for enhanced herbicide metabolism, contributing to herbicide resistance in sugarcane (Dermawan et al. 2016).
3. Glutathione S-transferase (GST) Enzymes (Herbicide Detoxification):
    - GSTs are another class of enzymes that can contribute to herbicide tolerance by conjugating herbicides to glutathione and facilitating their sequestration or removal from the plant (Kurama et al. 2002).
    - Markers for GST genes can be used to select plants with higher levels of herbicide-detoxifying enzymes, improving resistance to herbicides such as glufosinate and triazine.

4. Herbicide Sequestration Pathways (Transporter Proteins):
   - Herbicides are often sequestered in vacuoles or other plant compartments to prevent their toxic effects on cellular processes. Genes involved in this process include those encoding ABC transporters or tonoplast-localized transporters (Dermawan et al. 2016).
   - Molecular markers linked to these genes can be used to select sugarcane plants with better herbicide sequestration capabilities, enhancing their resistance to various herbicides (Dermawan et al. 2016).

23.8 Genomic Regions and QTL Mapping for Herbicide Resistance

Through genetic mapping and GWAS, researchers can identify genomic regions linked to herbicide tolerance (Meena et al. 2022). These regions often contain multiple genes that contribute to the overall resistance phenotype. By mapping the QTLs associated with herbicide tolerance traits, breeders can develop marker sets that allow for more efficient selection in breeding programs (Meena et al. 2022).

- Example: In a study of herbicide resistance in sugarcane, researchers have identified several QTLs associated with glyphosate resistance, which can be used for marker-assisted selection in breeding programs (Meena et al. 2022). These QTLs represent regions in the genome where genes involved in glyphosate resistance are likely located (Meena et al. 2022).

23.8.1 Benefits of Genomic Selection and Marker-Assisted Breeding for Herbicide Tolerance in Sugarcane

23.8.1.1 Accelerated Breeding Cycle

Both genomic selection and marker-assisted breeding reduce the time required to develop herbicide-resistant sugarcane varieties. Traditional breeding often requires multiple generations of phenotypic selection and extensive field trials. Still, using molecular markers, breeders can identify the best candidates for herbicide tolerance earlier in the breeding cycle (Sandhu et al. 2022).

23.8.1.2 Improved Precision and Efficiency

Molecular markers linked to herbicide resistance traits enable breeders to make more accurate selections, even without phenotypic expression. This results in a higher probability of success in developing varieties with the desired herbicide

tolerance, as breeders can select plants with favorable genetic profiles that are more likely to express the desired traits (Sandhu et al. 2022).

23.8.1.3 Reduced Need for Chemical Herbicide Applications

Herbicide-tolerant sugarcane varieties allow for better weed control, reducing the reliance on chemical herbicides. This improves the sustainability of sugarcane farming and helps mitigate the environmental impact of herbicide overuse. Herbicide-resistant sugarcane can also enhance growers' profitability by reducing herbicide costs and increasing yields (Hasan et al. 2021).

23.8.1.4 Enhancing Multiple Traits Simultaneously

Both genomic selection and marker-assisted breeding can be applied to select multiple traits simultaneously. Breeders can combine herbicide tolerance with other desirable characteristics, such as high yield, disease resistance, or drought tolerance, creating sugarcane varieties that are herbicide-olerant and agronomically superior (Hasan et al. 2021).

23.9 Future Perspectives

The future of herbicide tolerance breeding in sugarcane lies at the intersection of genetic innovation, sustainable agricultural practices, and technological advancements. As the global demand for sugar and biofuels continues to rise, addressing the challenges posed by weeds, limited labor, and environmental sustainability will be crucial for the future of sugarcane farming.

23.9.1 Advancements in Gene Editing Technologies

The rapid progress of gene editing technologies, particularly CRISPR-Cas9, presents an exciting avenue for developing herbicide-tolerant sugarcane varieties. These tools offer precise, targeted modifications that could introduce herbicide resistance without transgenic approaches, allowing for faster and more efficient breeding processes. Continued refinement of these technologies will expand their applicability, reducing the risks of off-target effects and improving the efficiency of creating herbicide-resistant crops.

23.9.2 Increased Focus on Sustainable Weed Management

As herbicide resistance continues to emerge in weed populations, there will be a growing emphasis on integrated weed management (IWM) strategies that combine herbicide tolerance with other biological, cultural, and mechanical control methods. Sugarcane breeding programs will likely focus on developing multitolerant genotypes that can withstand multiple herbicide classes and complement nonchemical control methods. This approach will reduce dependency on single herbicide modes of action, mitigating the risks associated with herbicide resistance.

23.9.3 Genomic Selection and Marker-Assisted Breeding

The advent of high-throughput sequencing technologies and the availability of sugarcane genomic resources will accelerate the identification of key genes involved in herbicide tolerance. Genomic selection and marker-assisted breeding are expected to play a pivotal role in developing herbicide-tolerant sugarcane varieties more efficiently, enabling the introduction of desirable traits in less time and at lower cost.

23.9.4 Environmentally Friendly Herbicides and Crop Protection Strategies

Developing more selective, environmentally friendly herbicides will complement breeding efforts aimed at herbicide tolerance. New herbicides with reduced environmental persistence and lower toxicity to non-target organisms could be integrated into future sugarcane farming systems, reducing the ecological impact of herbicide use.

23.9.5 Precision Agriculture and Mechanization

The integration of precision agriculture technologies, such as remote sensing, drones, and automated machinery, will enable more precise herbicide application and enhanced weed management. Herbicide-tolerant sugarcane varieties and mechanized weed control systems will facilitate more efficient, sustainable, and labor-saving farming practices, making large-scale sugarcane cultivation more economically viable.

23.9.6 Climate Change and Adaptation

Climate change is expected to alter weed dynamics, pest pressures, and growing conditions, potentially exacerbating the challenges in sugarcane production. Herbicide-tolerant genotypes will need to be developed with a focus on resilience to changing climatic conditions and their ability to thrive under varying environmental stresses (Verma et al. 2021, 2024c). Future breeding programs must prioritize herbicide tolerance and drought resistance, heat tolerance, and other traits that enhance sugarcane's adaptability to diverse climates.

23.10 Conclusion

Sugarcane, a critical crop for sugar and biofuel production, faces significant challenges from weed competition, which can drastically reduce the yield and quality of milling cane. Effective weed management, mainly through herbicide use, has become essential to modern sugarcane farming. However, the increasing reliance on herbicides has led to concerns over crop sensitivity, environmental impacts, and the development of herbicide-resistant weeds. So herbicide tolerance is an important trait in the genetic improvement of sugarcane. These challenges underscore the need for breeding sugarcane varieties with enhanced herbicide tolerance, which can help improve weed control while promoting sustainability in agricultural practices. While sugarcane's complex polyploid nature and long breeding cycle pose obstacles to traditional breeding methods, recent advances in genetic engineering, including the use of CRISPR-Cas9, offer promising solutions for developing herbicide-tolerant genotypes.

Moreover, innovations in genomic selection, integrated weed management, and precision agriculture are poised to accelerate further the breeding of sugarcane varieties that are both high-yielding and resistant to herbicides. The future of herbicide tolerance breeding in sugarcane is not only about developing more resilient varieties but also integrating these advancements into holistic, environmentally sustainable farming systems. The global sugarcane industry can address the twin goals of increased productivity and environmental stewardship by combining genetic improvements with better herbicide management practices and precision farming technologies. Continued research and collaboration across scientific and policy sectors will be essential to solve these goals and ensure the long-term success of herbicide-tolerant sugarcane cultivars for sustainable cane farming.

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Chapter 24
Recent Development of Sugarcane Breeding in Pakistan

Ghulam Sarwar Solangi

Abstract Sugarcane (*Saccharum officinarum* L.) is a high-value cash crop in Pakistan, making the country the fifth largest producer globally, with an annual production of approximately 67 million tons of cane. This chapter offers an overview of advancements in sugarcane breeding, the development of germplasm, and associated biotechnology within Pakistan. To date, variety development has primarily focused on the selection of imported seeds and clones. Although this approach has yielded success, further enhancements are necessary. This review delineates various breeding strategies utilized by research institutes and sugar mills, encompassing the historical context and contemporary methodologies of breeding programs, alongside significant germplasm development initiatives at both national and international scales. Genetically modified sugarcane varieties, such as CABB-HTS (herbicide-tolerant transgenic sugarcane) and CABB-IRS (insect-resistant transgenic sugarcane) have been approved for cultivation. It is concluded that full-scale breeding programs should be established to develop new sugarcane varieties that offer higher economic value for sugar and other products at a lower cost than existing commercial varieties.

Keywords Sugarcane · Bioenergy crop · Breeding · Research and Development · Pakistan

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24.1 Introduction

24.1.1 Sugarcane in the Economy of Pakistan

Sugarcane (*Saccharum officinarum* L.) belongs to the family Poaceae and basically consists of a stem and straw. The sugarcane straw (or trash) is divided into three principal components: fresh leaves, dry leaves, and tops. The sugarcane stem is milled to obtain the cane juice, which is subsequently used for sugar (sucrose) or alcohol (ethanol) production (Afghan et al. 2024). The residual fraction from the sugarcane stem milling is named bagasse. Sugarcane bagasse (SB) and sugarcane straw (SS) are normally burned in industries to supply all the energy required in the process. If, instead, both were used for ethanol production, much more ethanol would be produced from each hectare of sugarcane processed. Sugarcane is significantly important to Pakistan's economy (Afghan et al. 2022). As one of the primary cash crops, sugarcane is vital for both rural livelihoods and national revenue (Kaahwa et al. 2023). Its cultivation is predominantly concentrated in the Punjab, Sindh, and Khyber Pakhtunkhwa provinces, where the climatic conditions are favourable for its growth.

The sugarcane industry is instrumental in supporting the agricultural sector (Li et al. 2024), employing millions of labourers engaged in its cultivation, harvesting, and processing in 89 sugar mills. Currently, Pakistan has 89 operational sugar mills, with 45 located in Punjab, 37 in Sindh, and 7 in Khyber Pakhtunkhwa. Moreover, the sugar mills, which process sugarcane into sugar and other by-products, serve as vital nodes for rural economic activity. These mills not only generate direct employment but also stimulate ancillary industries, including transportation, packaging, and fertilizer production. In 1947, coinciding with Pakistan's independence, the cultivated area for sugarcane was approximately 200,000 ha (Chullen 2002). The majority of the cane was locally processed into noncentrifugal sugar varieties known as 'gur' and 'khand', or 'shakar'. The expansion of processing capacity began in 1949 with the establishment of the Premier Mill in Mardan. From 1959 to 1990, a total of 28 new mills were constructed, followed by the establishment of an additional 31 mills between 1990 and 1998 (Chullen 2002). The sugarcane harvesting season typically occurs from November to March, while beet sugar is harvested from May to June. Sugarcane production forecast during 2024–25 remains unchanged at 83.5 million metric tons, which is 2% higher than the 2023–24 estimate. This increase in production is attributed to a rise in the cane area. In Pakistan during 2023–24, sugarcane cultivation covered approximately 1.2 mha, reflecting a decrease of 10.5% as compared to last year 1.3 mha. This crop not only contributes significantly to the economy but also accounts for 0.8% of the Gross Domestic Product (GDP) and 3.5% of the overall agricultural value addition.

Furthermore, sugarcane production was slightly declined by 0.4%, resulting in an output of 87.6 mts in 2023–24 compared to 88.0 mts last year. Sugarcane area, yield, and production in Pakistan over the last 5 years are presented in Table 24.1. Over the past two years, sugarcane has proven to be more profitable than competing

Table 24.1 Sugarcane area, yield, and production in Pakistan

Year	Area (1000 ha)	Production (1000 Tons)	Yield (kg/ha)
2019–2020	944	66,380	63,841
2020–2021	883	81,009	69,534
2021–2022	862	88,651	70,341
2022–2023	843	87,981	66,711
2023–2024	794	87,638	74,252

crops, such as maize and cotton. The sugar sector is classified as a vital industry due to its linkages with various other sectors, such as agriculture, food processing, and export (Huang et al. 2022; Li et al. 2023). It is worth noting that Pakistan is among the top sugarcane-producing countries globally, with production levels consistently exceeding 60 mts annually. This output supports both domestic consumption and export opportunities, thus enhancing foreign exchange earnings. However, the sugarcane industry in Pakistan faces several challenges that need to be addressed to ensure its sustainability and growth. These include fluctuating market prices, inefficient production practices, and outdated technology. Additionally, water scarcity and climate change impact the availability of resources essential for sugarcane cultivation, threatening productivity levels (Verma et al. 2024a, b, c).

24.2 Sugarcane Breeding

The crop known as sugarcane belongs to the genus *Saccharum* L., which is a part of the Poaceae family. This genus encompasses 6 different species characterized by varying sizes and chromosome counts: *S. officinarum*, *S. spontaneum*, *S. robustum*, *S. barberi*, *S. sinense*, and *S. edule*. Additionally, 4 genera, i.e., *Sclerostachya*, *Miscanthus*, *Narenga*, and *Erianthus*, are closely related to *Saccharum* L. and possess the capacity to interbreed, thereby forming the *Saccharum* complex.

24.2.1 Sugarcane Breeding in Pakistan

In Pakistan, sugarcane breeding is a significant area of agricultural research and development, aimed at improving sugarcane varieties to enhance sugar yield, resistance to diseases and pests, and adaptability to changing climatic conditions. One of the primary challenges faced by taxonomists and molecular biologists is the high level of polyploidy and aneuploidy observed in genera of the '*Saccharum* complex', which is characterized by an unbalanced number of chromosomes. Therefore, the limitations in genetic improvement can be attributed to the complexity and size of the sugarcane genome (Pandey et al. 2011). One of the desirable characteristics of a sugarcane cultivar is excessive nonflowering, as flowering induces the pithing

process within the stalks. The significance of flowering as a crucial characteristic in a breeding program necessitates the establishment of breeding stations in specific locations where these phenotypes can regularly bloom and exhibit fertility (Sica 2021).

24.2.2 Importance of Sugarcane in Pakistan

- Sugarcane is a major cash crop and a key source of the raw material for the sugar industry in Pakistan.
- It contributes significantly to the agricultural GDP and supports livelihoods in rural areas.

24.2.3 Major Sugarcane-Producing Provinces of Pakistan

Sindh, Punjab, and Khyber Pakhtunkhwa provinces of Pakistan are the principal regions for sugarcane cultivation.

24.3 Objectives of Sugarcane Breeding

24.3.1 Higher Yield

The primary objective of sugarcane breeding is to achieve a higher yield, which is essential for enhancing the productivity and profitability of sugarcane cultivation. By selecting and crossbreeding superior varieties with desirable traits, breeders aim to develop cultivars that produce greater biomass and sugar content (Liang et al. 2024). This endeavour not only addresses the increasing global demand for sugar but also contributes to the sustainability of agricultural practices by optimizing resource use and reducing the environmental impact of sugarcane production. Ultimately, improved yield from breeding programs is a critical factor in ensuring food security and supporting economic growth in sugarcane-producing regions (Verma et al. 2022a; Wu et al. 2022).

24.3.2 Improved Sugar Recovery

The primary objective of sugarcane breeding is to enhance sugar recovery, thereby increasing the efficiency and profitability of sugar production. This involves the selection and development of cane varieties with superior sucrose content, improved vigour, and resilience to pests and disease (Singh et al. 2019; Verma et al. 2022b). By optimizing these traits through targeted breeding programs, researchers aim to create cultivars that not only yield higher sugar quantities but also adapt to varying environmental conditions. Ultimately, the achievement of improved sugar recovery is essential for meeting the growing global demand for sugar while promoting sustainable agricultural practices (Oliveira et al. 2022).

24.3.3 Resistance to Pests/Diseases

The objectives of sugarcane breeding encompass a crucial focus on enhancing resistance to pests and diseases. This endeavour seeks to develop cultivars that exhibit improved resilience against common agricultural threats, thereby reducing the reliance on chemical control measures. By employing genetic selection and biotechnological advancements, breeders aim to elevate the inherent defenses of sugarcane plants, ultimately contributing to increased yield stability and sustainable production practices. The successful integration of pest and disease resistance not only bolsters farmer productivity but also supports environmental sustainability within the sugarcane industry (Song et al. 2019).

24.3.4 Drought Tolerance

- *Efficient Water Use:* Develop varieties with improved water-use efficiency to sustain growth with minimal water.
- *Deep Root System:* Select deeper and more extensive root systems for better water absorption.
- *Stomatal Regulation:* Enhance the ability to regulate stomatal opening, reducing water loss.
- *Photosynthetic Efficiency:* Improve the ability to photosynthesize efficiently under water-limited conditions.
- *Early Maturity:* Develop early maturing varieties to escape prolonged drought periods.
- *High Biomass and Yield Stability:* Ensure that yield and biomass production remain stable despite drought stress.

24.3.5 Salinity Tolerance

- *Salt Exclusion Mechanisms:* Select varieties that can prevent excess salt uptake through roots.
- *Ion Homeostasis:* Improve the ability to regulate and compartmentalize harmful ions, such as sodium (Na^+) and chloride (Cl^-).
- *Osmotic Adjustment:* Enhance the production of osmoprotectants (e.g., proline and glycine betaine) to maintain cell turgor under saline stress.
- *Salt-Tolerant Root Systems:* Develop roots that perform well in saline soils without losing functionality.
- *High Yield and Quality in Saline Conditions*: Maintain high sucrose content and cane yield despite high soil salinity.
- *Compatible Symbiotic Relationships:* Enhance association with salt-tolerant microorganisms to improve soil conditions.
- *High Yield Potential:* Developing varieties with higher cane and sugar yield per hectare.
- *Late Maturity:* Developing varieties that maintain high sugar content for late harvesting.
- *High Sugar Recovery:* Enhancing sucrose content to improve sugar extraction.
- *Lodging and Frost Resistance:* Ensuring sturdier varieties that can resist lodging and frost damage.
- *Ratooning Ability:* Improving the ability to produce multiple ratoon crops with good yield and quality.
- *Adaptability:* Creating varieties suited to different agro-climatic conditions and soil types.
- *Juice Quality Improvement:* Enhancing juice purity and reducing impurities for efficient sugar processing.

24.3.6 Early and Late Varieties of Sugarcane

Sugarcane varieties are categorized based on their maturity period as early maturing varieties (harvested in 10–12 months) and late-maturing varieties (harvested in 12–16 months). These varieties continue to accumulate sucrose for a longer time, making them suitable for extended harvesting.

24.4 Research Institutions Involved

24.4.1 Sugarcane Research Institute, Faisalabad

Established in 1934 in Lyallpur, the Sugarcane Research Station was upgraded in 1978 to the Sugarcane Research Institute, Faisalabad. The primary focus of the research has been the development of high-yielding cane and sugar varieties that are resistant to diseases and insect pests, along with the advancement of improved production technology. The Annual Research Program is formulated to outline and develop the research strategy for the upcoming crop year. The Research Program encompasses ongoing trials and experiments across various disciplines, including sugarcane breeding, pathology, entomology, agronomy, and technology, along with the introduction of new experiments in the current research year. The components of the sugarcane breeding program include the collection of fuzz and cultivars, the raising and selection of seedlings, and the screening and selection of clones at various stages, along with their varietal adaptability under diverse soil and climatic conditions. Additionally, the research efforts involve studying cane flowering at the Research Sub Station in Pail and Charapani, Murree.

24.4.2 Nuclear Institute of Agriculture, Tandojam

Nuclear Institute of Agriculture (NIA), Tandojam, is a leading agricultural research institute in Pakistan, specializing in the development of improved crop varieties, including sugarcane. NIA operates under the Pakistan Atomic Energy Commission (PAEC) and employs nuclear and biotechnological techniques to enhance agricultural productivity. NIA has made significant contributions to sugarcane breeding by developing high-yielding, disease-resistant, and climate-resilient varieties. Some key aspects of its sugarcane research include:

Mutation Breeding: Using radiation-induced mutations to improve sugarcane traits, such as yield, sucrose content, and disease resistance against red rot and smut.

Hybridization Programs: Crossbreeding different sugarcane genotypes to develop superior cultivars suited to Pakistan's climate.

Biotechnological Applications: Utilizing tissue culture and molecular markers to speed up breeding and disease resistance screening.

Improved Agronomic Practices: Researching efficient water management, fertilizer use, and pest control to optimize sugarcane production.

24.4.2.1 Impact on Sugarcane Industry

NIA's research has helped introduce high-yielding sugarcane varieties that contribute to increased sugar production and profitability for farmers. These advancements support Pakistan's sugar industry and reduce reliance on imported sugar. With continued research and collaboration, NIA, Tandojam, remains a key player in strengthening Pakistan's sugarcane sector through scientific innovation and sustainable agricultural development.

24.4.3 Sugarcane Research Institute, Tandojam

The Sugarcane Research Institute (SRI), Tandojam, is a prominent agricultural research centre in Pakistan, focusing on the development and improvement of sugarcane varieties. Established in 1967–68, the institute has been instrumental in advancing sugarcane cultivation practices and enhancing crop productivity in the region.

24.4.3.1 Varietal Development

SRI Tandojam has been involved in screening and developing promising sugarcane genotypes suitable for the agroecological conditions of Sindh. Field experiments conducted during the 2016–2018 cultivation seasons evaluated seventeen sugarcane genotypes to identify those with superior agronomic and quality traits.

24.4.3.2 Nutrient Management Research

The institute has conducted studies on the impact of various fertilization strategies on sugarcane growth and yield. For instance, research on the effect of different potassium levels on yield and sugar recovery has provided valuable insights into optimal fertilization practices for local sugarcane varieties.

24.4.3.3 Educational Initiatives

SRI Tandojam collaborates with academic institutions to provide practical training opportunities. In 2023, the institute facilitated internships for students from Sindh Agriculture University, Tandojam, offering hands-on experience in sugarcane research and cultivation techniques.

24.4.3.4 Knowledge Dissemination

The institute organizes events, such as the "Sugarcane Traveling Seminar," which serves as a platform for scientists and researchers to observe field conditions, share data, and discuss advancements in sugarcane research. Through these initiatives, the Sugarcane Research Institute in Tandojam continues to play a vital role in supporting the sugarcane industry in Pakistan by promoting sustainable agricultural practices and fostering innovation in crop management.

24.4.4 Shakarganj Sugar Research Institute, Jhang

The Shakarganj Sugar Research Institute (SSRI), established on July 13, 1983, is a pioneering private-sector initiative in Pakistan dedicated to advanced sugarcane research. Located 3 km along Toba Tek Singh Road in Jhang, Punjab, SSRI operates under the umbrella of Shakarganj Limited, a company incorporated in 1967 and listed on the Pakistan Stock Exchange.

24.4.4.1 Varietal Development

SSRI focuses on breeding new sugarcane varieties that offer higher yields and improved sugar content. Through its research, the institute has developed proprietary sugarcane varieties that have enhanced crop productivity by approximately 10% for Shakarganj's cane growers.

24.4.4.2 Biological Pest Control

The institute emphasizes environmentally friendly pest management strategies. It has developed efficient biocontrol systems to manage sugarcane pests, promoting sustainable agriculture.

24.4.4.3 Research Collaboration

SSRI collaborates with institutions, such as the Sugarcane Research Institute in Faisalabad to exchange germplasm and conduct varietal trials. This partnership aims to evaluate and select promising sugarcane genotypes under diverse environmental conditions.

24.4.4.4 Knowledge Dissemination

The institute publishes the "Pakistan Sugar Journal," contributing to the dissemination of research findings and advancements in sugarcane technology. Through these initiatives, SSRI continues to play a vital role in advancing sugarcane research and supporting the agricultural sector in Pakistan.

24.4.5 Sugar Crops Research Institute, Mardan

The Sugar Crops Research Institute (SCRI) in Mardan, Khyber Pakhtunkhwa, Pakistan, was established in 1952 and upgraded to an institute in 1981. Its mandate includes the development of high-yielding, disease-resistant, and high-quality varieties of sugarcane and sugar beet, as well as the creation of improved production technologies and their dissemination to growers through adaptive research programs.

24.4.6 National Agricultural Research Centre (NARC)

The National Agricultural Research Centre (NARC) is the premier research establishment of the Pakistan Agricultural Research Council (PARC), established in 1984. It is located near Rawal Lake, approximately six kilometres southeast of Islamabad, covering an area of approximately 1396 acres. NARC also features one of the largest agricultural libraries in Pakistan, offering a vast collection of books, journals, and research papers related to agriculture and allied fields. The library is open to the public, including researchers, students, and farmers, and provides access to online databases, e-books, and other resources.

24.4.7 Pakistan Agricultural Research Council (PARC), National Sugar and Tropical Horticulture and Research Institute (NSTHRI), Thatta

The Pakistan Agricultural Research Council (PARC) is a key institution in Pakistan responsible for agricultural research, promoting innovation in agricultural practices and enhancing the country's agricultural productivity. It operates under the Ministry of National Food Security and Research. The National Sugar and Tropical Horticulture Research Institute (NSTHRI) is one of PARC's research entities. Located in Thatta, the institute focuses on sugarcane and tropical horticultural research. It aims to improve the productivity and sustainability of these crops, essential to Pakistan's agricultural economy. NSTHRI researches improving crop

varieties, pest management, irrigation techniques, and other agricultural practices specific to sugarcane and tropical fruits.

24.4.8 Provincial Agriculture Research Centres and Private Farms

In Pakistan, sugarcane breeding is a collaborative effort involving Provincial Agricultural Research Centres and private farms, each playing a distinct yet complementary role in developing high-yielding, disease-resistant, and climate-adapted sugarcane varieties.

24.4.9 Role of Provincial Agricultural Research Centres

Provincial research centres operate under provincial agriculture departments and focus on:

- *Developing and Testing New Varieties:* Conduct breeding programs to develop high-yield, disease-resistant, and climate-resilient sugarcane varieties.
- *Germplasm Conservation:* Maintain genetic resources and introduce new sugarcane germplasm for crossbreeding.
- *Disease and Pest Management:* Conduct research on sugarcane pests (e.g., borers and whiteflies) and diseases (e.g., red rot and smut).
- *Farmer Training and Technology Transfer:* Educate farmers on improved farming techniques, water conservation, and pest control.
- *Collaboration with PARC and International Institutes:* Work with national and global research bodies to improve breeding techniques.

24.4.10 Role of Private Farms in Sugarcane Breeding

Private farms, including sugar mills, corporate farms, and progressive farmers, contribute by:

- *Field Trials and Adaptive Research:* Conduct trials of new sugarcane varieties to assess performance under different agro-climatic conditions.
- *Seed Multiplication and Distribution:* Produce and distribute certified disease-free sugarcane seed to ensure high-quality planting material.
- *Adoption of Modern Breeding Techniques:* Use biotechnology, tissue culture, and precision farming to improve yield and sugar recovery.

- *Investment in Research and Development:* Some sugar mills invest in research partnerships to develop high-yielding and high-sucrose varieties.
- *Farmer Support Programs:* Provide farmers with financial incentives, training, and advisory services to improve cultivation practices.

24.4.11 Collaboration Between Public and Private Sectors

Successful sugarcane breeding in Pakistan requires strong public-private partnerships. Private farms benefit from government research and improved varieties, while research centres get real-world feedback from private farms for further refinement.

24.5 Breeding Techniques

- *Conventional Breeding:* Cross-pollination between selected parent plants for desired traits.
- *Mutation Breeding:* Inducing genetic changes using radiation or chemicals.
- *Biotechnology Approaches:* Genetic engineering and molecular markers to speed up breeding processes.
- *Selection Breeding:* Screening local and exotic germplasm for superior traits.

24.5.1 Challenges in Sugarcane Breeding

- *Climatic Changes:* Adverse impacts of climate variability on yield and disease prevalence.
- *Pest and Disease Pressure:* Lack of sustainable resistance in some varieties.
- *Water Scarcity:* Limited water resources affect productivity.
- *Limited Resources:* Insufficient funding and technology in some research centres.

The commercial sugarcane breeding program in Pakistan remains underdeveloped, primarily relying on selection breeding methodologies. A commercial variety, HSF-240, was released in 2002 and quickly became dominant, accounting for 32% of the Punjab cultivation area during the 2016–17 season. However, its prevalence significantly declined to 10% by 2018–19 due to susceptibility to smut, despite initially being resistant to major diseases until 2013. This decline is attributed to the mutation of the sugarcane smut pathogen and the use of smut-infected seed cane from ratoon crops for planting (Afghan et al. 2019). Another instance of significant variety loss is observed with L-116, which was once a staple in southern Punjab and upper Sindh for nearly two decades but later became susceptible to red rot. Similarly, the variety BF-162 (B = Barbados fuzz and F = Faisalabad selection) has recently

exhibited marked susceptibility to rust and moderate susceptibility to red rot. Previously, a dominant cultivar in southern Punjab covered nearly 75% of the production area and enabled sugar mills to achieve the highest sugar recoveries in the province (Malik 2000). The decline of this variety may be attributed to the use of infected seed cane for commercial planting. Moreover, BF-162 has shown a lack of resistance to newly virulent strains of red rot, which are now widespread in southern Punjab.

Nationally, a range of research and funding bodies invest in sugarcane development, primarily including the Sugarcane Research and Development Board (SRDB), Punjab Agriculture Research Board (PARB), Agriculture Linkage Program (ALP), Pakistan Agricultural Research Council (PARC), and Science Foundation and Higher Education Commission (HEC). Additionally, three private organizations funded by sugar companies are independently conducting sugarcane research. Current limitations in sugarcane breeding include insufficiently controlled temperature and light facilities for inducing flowering, as natural conditions conducive to good flowering are lacking (Zaidi 2016). Consequently, investments in variety development are predominantly directed towards the importation of true seed (fuzz) and clones/varieties, alongside the selection of locally adapted clones from these imports. Pakistan currently maintains germplasm and variety exchange agreements (free of cost) with ARS-USDA, Sri Lanka, Reunion (France), and Barbados.

The development of agreements between the Sugarcane Research and Development Board (SRDB), Punjab, Pakistan, and the ARS-USDA as well as Brazil for the purchase of germplasm, along with agreements with China and Thailand for free access is in progress. Historically, sugarcane institutes in Pakistan have acquired germplasm from BSES and CSR Australia, ARS-USDA Houma and Canal Point breeding stations, and West Indies Central Sugarcane Breeding Station Inc., Barbados. The selection program primarily utilizes fuzz imported from São Paulo (Brazil), Canal Point (USA), Natal (South Africa), Uda Walawa (Sri Lanka), and Barbados.

Seedling populations derived from the local and imported fuzz undergo mass selection, with clones evaluated for yield and disease tolerance through various selection stages (Cheavegatti-Gianotto et al. 2011). Field trials are conducted across different agro-climatic regions in each province to assess the adaptability of varieties to diverse ecological conditions (Moore et al. 2013). Currently, dozens of varieties have reached advanced selection stages, with approximately 50% of commercial varieties in Pakistan originating from fuzz imported from Canal Point.

24.5.2 Parental Selection Methods of Breeding

The selection of a parental clone primarily hinges on the expression of its characteristics. In Pakistan, the criteria for parental selection encompass factors, such as cane yield, sugar content, maturity trends (early, medium, and late), salinity tolerance, lodging resistance, and ratoon ability (Moore et al. 2013). The other criteria also

encompass resistance to various diseases including red rot (*Colletotrichum falcatum*), smut (*Sporisorium scitamineum*), Pokkah Boeng (*Fusarium moniliform*), and sugarcane mosaic virus. Additionally, tolerance for borer complex (top borer *Scirpophaga nivella*, stem borer *Chilo terrenellus*, root borer *Polyocha depressella,* and Gurdaspur borer *Bissetia steniella*), white fly (*Aleurolobus barodensis*, *Pyrilla perpusilla*, and termites *Odontotermes obesus*), and black bug (*Cavelerius excavates*) is also considered significant (Munir et al. 2020).

In 2014, a collaborative project was initiated between Pakistan and Sri Lanka to evaluate parental combinations from both sides. Since 2016, Pakistan has been receiving fuzz from crosses conducted in Sri Lanka as part of this initiative. To date, this project has demonstrated success, fostering additional efforts in various breeding stages in both countries, along with facilitating the training and exchange of scientists. In Pakistan, flower induction for breeding occurs in the mountainous regions of Punjab, Khyber Pakhtunkhwa, and the coastal areas of Sindh. During the 2019–20 period, the Sugarcane Breeding Sub-Station Murree (Sugarcane Research Institute, Faisalabad) produced 84 crosses. Additionally, the National Sugar Crops Research Institute, Thatta, Sindh produced 60 crosses, while the Sugarcane Breeding Station, Jabban Dargai, Khyber Pakhtunkhwa produced 75 crosses.

24.6 First, New Genetically Modified (GM) Sugarcane Varieties Approved as Food Crop

Pakistan has green lighted commercial farming of its inaugural genetically modified (GM) food crop, granting authorization for two varieties of sugarcane varieties developed by the University of Agriculture, Faisalabad (UAF), Punjab. The Technical Advisory Committee (TAC) under the auspices of the federal Environment Protection Agency (EPA) sanctioned the commercialization of two high-yielding GM sugarcane varieties: CABB-HTS, an herbicide-tolerant variety and CABB-IRS, an insect-resistant transgenic sugarcane seed. This marks the first GM sugarcane as a food crop being approved for large-scale cultivation in the country, deviating from previous uses of GM crops in Pakistan, such as *Bacillus thuringiensis* (Bt) cotton was used only for oil extraction and animal feed.

The recent approval of GM sugarcane varieties promises significant advancement in agricultural efficiency and productivity. One such variety is engineered for resistance against pest attacks by the insertion of a nonplant toxic gene, specially utilizing *Cry* proteins derived from *Bacillus thuringiensis* to effectively manage lepidopteran insect pests. Furthermore, the second GM variety demonstrates tolerance to nonselective herbicides, including Glyphosate and Glufosinate, which facilitates effective weed control. Yield estimates from field trials across several locations

in Punjab and Sindh reveal a noteworthy potential for increased productivity. Notably, these new varieties are expected to eliminate the costs associated with insecticide application, thus enhancing input efficiency due to inherent insect resistance. Last year, the university asserted that the CABB-IRS and CABB-HTS sugarcane varieties could yield significant economic benefits by decreasing reliance on granular pesticides for borer control, which typically incurs expenses of US$ 31–35/ha. The introduction of these engineered sugarcane varieties is poised to mitigate losses attributed to weeds and insects, which can account for up to 25 and 40% reduction in yield, respectively. Consequently, the development of these varieties is anticipated to lead to a substantial increase in sugarcane yield per hectare, making a pivotal advancement in agricultural biotechnology and sustainable farming practices.

Additionally, the Pakistan-US Science and Technology Cooperation Program initiated a project to enhance sugarcane production through modern breeding technologies. This collaboration underscores the importance of international partnerships in advancing agricultural research and development in Pakistan. Pakistan has imported sugarcane germplasm from many countries, including the United States, the Agriculture Research Service in Canal Point, USA, Sao Paul, Brazil, and SRI, Sri-Lanka, Sri Lanka. Some of the popular varieties of sugarcane that have been imported to Pakistan include CP 43-33, CP 72-2086, CP 77-400, CPF-237, CPF-246, CPF-248, HSF-240, SPF-213, SPF-234, and SPF-245. The Sugarcane Research Institute in Faisalabad has also imported sugarcane fuzz from Brazil, Australia, and South Africa. The fuzz was then sown at the institute and the Shakarganj Sugar Research Institute in Jhang.

24.6.1 Prominent Varieties

Several improved sugarcane varieties have been developed in Pakistan, such as CPF-246, HSF-240, CP-77-400, and CPF-237. These varieties are known for high yield, good sugar recovery, and resistance to common diseases.

24.6.2 Mills Refuse to Buy 12 Cane Varieties

Sugar mills in the district Muzaffargarh, Punjab, Pakistan, have refused 12 types of cane varieties from the mills premises due to short of the standard weight and juice quality. The banned varieties were NSG 59, US 272, US 658, SP 238, AUS 133, SP 93, US 133, US 127, US 778, AUS 138, CSSG 676, and 302/235.

24.7 Conclusion and Future Research

Sugarcane breeding in Pakistan plays a crucial role in enhancing crop productivity, disease resistance, and environmental adaptability. Given the country's reliance on sugarcane for its sugar industry and by-products, breeding efforts focus on developing high-yielding, disease-resistant, and climate-resilient varieties. Despite challenges, such as water scarcity, pest infestations, and limited research funding, advancements in biotechnology and conventional breeding methods have improved genetic diversity. Collaboration between research institutions, universities, and farmers is essential to further enhance sugarcane breeding programs. By investing in modern breeding techniques and sustainable agricultural practices, Pakistan can ensure a stable and prosperous sugarcane industry, contributing to food security and economic growth.

With new challenges in sugarcane cultivation, all future breeding programs must focus on ecologically viable, environment-friendly, and resource-matching technologies. Bioethanol production from sugarcane will be the major focus in the coming years to meet the energy demands of Pakistan.

- Biotechnology Integration: Wider use of genetic tools, such as CRISPR and molecular markers.
- Climate-Resilient Varieties: Breeding for heat and drought tolerance.
- Collaboration: Strengthening partnerships between research institutions, sugar mills, and farmers.
- Policy Support: Government initiatives to fund research and ensure technology transfer.

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Chapter 25
Sugarcane Clean Seed Production: Technological Development, Practical Application, and Commercialization

Aqsa Parvaiz, Muhammad Saeed, Faiz Ahmad Joyia, Saba Zafar, Aneela Shadab, Muhammad Sarwar Khan, and Ghulam Mustafa

Abstract The production of clean seed in sugarcane farming is crucial for attaining robust, disease-resistant crops with elevated yield potential. This chapter analyzes technical innovations, practical implementations, and marketing tactics pertaining to clean seed production in sugarcane. This research starts with examining critical technologies, including tissue culture, thermotherapy, and molecular diagnostics, which facilitate the generation of a clean, pathogen-free planting material. This discourse centers on pragmatic techniques for producing clean seeds, including nurseries, quarantine protocols, and controlled environment propagation. This chapter highlights commercial elements, outlining the need for an organized supply chain, quality control systems, certification requirements, and the roles of government and private sector collaborations in improving clean seed production. This chapter provides an in-depth analysis of the science and commercialization of clean seed production, aiming to enhance knowledge and encourage the implementation of novel approaches to promote sustainable sugarcane agriculture.

Keywords Sugarcane · Seed production · Conventional tissue culture approach · Temporary impression bioreactor system · Pathogen-free seed

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25.1 Introduction

Approximately 70% of the world's sugar production comes from sugarcane crushing and processing. Worldwide, sugarcane is a significant player in the sugar industry. The use of sugarcane as a primary ingredient in bioethanol production has grown in recent years. Animal feed, antibiotics, particle boards, bio-fertilizers, and raw materials for energy production are just a few of the numerous things that may be made from sugarcane (Li et al. 2024; Chen et al. 2020, 2024). Most countries are considering renewable plant-based energy alternatives due to dwindling petroleum supplies. Bioethanol production from sugarcane is attracting a lot of attention from countries worldwide (Verma et al. 2023a, 2024a, b). The incidence of diseases is one factor limiting industrial and chewing sugar cane production in Nigeria. Limited and late allocation of farms, limited finance, and poor cane production are the other key restrictions that have been highlighted. More critically, there is a persistent need to achieve outstanding agronomic performance with long-term resistance to diseases and pests (Malviya et al. 2020; Oliveira et al. 2022). More critically, there is a persistent need to achieve outstanding agronomic performance with long-term resistance to diseases and pests (Li et al. 2023; Verma et al. 2024c). Sugarcane cultivars that are high-yielding, disease-resistant, prolific, and resistant to pests and diseases should be made available to solve the problem of poor cane yield (Song et al. 2019; Singh et al. 2019). As a result of the low seed multiplication rate of 1:6 to 1:8 that occurs when sugarcane is propagated using vegetative methods, the seed production of newly released varieties is generally delayed. Throughout several cycles of field production, the seed takes on a variety of illnesses and infections. Due to this, the lack of availability of the disease-free planting material that is true to type is a significant barrier to improving sugarcane yield (Dukoon et al. 1995; Zheng et al. 2023).

25.2 Importance of Clean Seed in Sugarcane Production

Several significant advancements in sugarcane biotechnology have been described. These advancements have been made to overcome the limitations that have been encountered in the process of generating cultivars that are more productive and helpful. Biotechnology has several prospects for the enhancement of sugarcane crop production (Verma et al. 2020a, b; Huang et al. 2022, 2024). More critically, there is a continuing need to develop commercially exploited clones with higher agronomic performance and long-term resistance to diseases and pests. This resulted in a significant amount of research being conducted in several scientific fields of biotechnology that are relevant to sugarcane breeding and disease management (Girei and Giroh 2012; Guo et al. 2021, 2023).

The scope and velocity of the biotechnology revolution have started to touch on lives in so many different ways that it looks like society is heading into the era of

biotechnology. Building the capacity for research and advancements in biotechnology is now being done in Nigeria and other developing nations in Africa and beyond. This is in contrast to other developed and developing countries, where it is a rising business that offers exceptional prospects in the years to come in terms of economics, society, and the environment. For example, it was claimed that sugarcane farmers achieved an average increase in production of 28 tons per hectare by only switching from traditional planting materials to planting materials grown from tissue culture plantlets.

25.3 Overview of Clean Seed Production Technologies

There is a wide range of sizes for sugarcane germplasm collections in Brazil. These collections are comprised of species belonging to the *Saccharum* complex, commercial cultivars, and elite clones with superior breeding characteristics. Over time, several attempts have been made to classify accessions as the primary factors that contribute to economic worth and encourage improvement. On the contrary, these efforts are quite complicated, and many accessions still need to be described to make them more suitable for breeding purposes. This process is carried out at a variety of improvement facilities. There are now five germplasm collections, all of which are situated in the northeastern region of Brazil. This region is specifically chosen because it provides good climatic conditions for the abundant blossoming of sugarcane and the generation of viable pollen. The RIDESA (RB) programs are served by two of these, the CTC program and the IAC program, with the latter being solely devoted to the breeding of energy cane. Despite the progress made in sugarcane breeding, practically all of the varieties planted across the globe are derived from a small number of parent plants (Fig. 25.1). This may be attributed to the fact that the genetic basis of current clones seems to be narrowing. This aspect, in conjunction with the intricate sugarcane genome, has hampered the progress of breeding efforts (Rahman et al. 2016).

25.4 Germplasm Collection and Development

Over the course of many decades, a number of expeditions were carried out, which ultimately led to the admission of a wider variety of species into two global collections: one in India and another in the United States of America. The purpose of these collections is to provide breeders with access to genetic reservoirs that may be used to develop new cultivars that are more resistant to diseases and pests and more productive (Afghan et al. 2024). As a result of these accessions, several programs have been engaging in the process of introgression since the 1960s. These programs aim to broaden the genetic foundation of their breeding populations and, as a result,

Fig. 25.1 Production data of sugarcane in different regions

enhance the genetic diversity of their offspring for specific characteristics (Sani et al. 2012).

As a result of the increased need for renewable energy sources, there has been an increase in interest in biomass (Liang et al. 2024), but sugar has not received a significant amount of attention. During the 1980s in Brazil, several programs selectively picked clones with a large biomass. Still, the usual selection method did not accept them because they had low sucrose and high fiber content. Although these efforts were effective in persuading the sugarcane sector of the significance of this technique for choosing and growing sugarcane that is more productive and fibrous, they were unsuccessful.

It has been observed throughout the last thirty years that owing to the genetic difference that exists between the *Saccharum* genus and *Erianthus,* less than thirty percent of the accessions of Erianthus are capable of producing viable seeds, mainly when they are employed as sources of pollen. When Erianthus is used as a female parent, the seedlings produced are often apomictic and may be immediately recognized by the lack of dewlap (Viswanathan 2016).

These hybrids and accessions have introduced and developed new energy canes. Since then, several efforts using wild species, particularly *S. spontaneum*, have begun. These operations have directed selection toward plants resistant to disease and have a high fiber and a low sucrose content but a high biomass output. The primary operations carried out in Brazil to acquire energy canes were established by firms no longer in operation. These companies include Canavialis, Vignis, and AGN Bioenergy. At this time, these initiatives are still being carried out by IAC and RIDESA with reduced effort, while BioVertis/GranBio provides their entire attention to the endeavor.

The Ministry of Agriculture, Livestock, and Supply has registered and protected eleven energy cane cultivars that BioVertis/GranBio produced under the abbreviation Vertix. These cultivars are now in the beginning stages of multiplication by the enterprises that are involved in the field. Recent efforts toward introgression led to the registration and protection of these cultivations. It is important to note that the success of this approach will not be reached until the following years when there will be a broader commercial acceptance of the strategy as well as the introduction of technologies of the second generation into the market (Sawant et al. 2014).

25.5 Technological Developments in Clean Seed Production

25.5.1 Sugarcane Production-Related Impacts

Increasing global sustainability while simultaneously satisfying the need for energy has been suggested as a potential option by cultivating bioenergy crops. Several studies have brought attention to the environmental advantages of sugarcane ethanol as a viable alternative for reducing greenhouse gas emissions compared to other biofuel feedstocks (Joshi et al. 2022, 2023; Verma et al. 2023b, c). The purpose of this article is to outline the generally analyzed characteristics (such as energy balance, greenhouse gas savings, biofuel yield, and water footprint) and to compare the primary energy crops (such as maize, sugarcane, wheat, sugar beet, and sorghum) that are used to generate bioethanol throughout the globe. This analysis is based on research that was conducted on life cycle assessment.

25.6 Tissue Culture Techniques and Their Role in Seed Production

25.6.1 Conventional Tissue Culture

There is now widespread recognition that micropropagation technology may be used to produce high-quality seeds. The continued high sugar output per unit area depends on the ongoing supply of an appropriate amount of high-quality seed cane. This seed cane must be genetically pure, free from diseases and pests, and free of nutritional problems. The use of tissue culture methods is the only way to accomplish this goal. As a result of the absence of any illnesses in the plants, the newly developed variety has been able to preserve its original vitality (Visessuwan et al. 1995). As a vegetatively propagated crop, sugarcane typically takes seven to eight years, or even longer, for a newly produced variety to spread to an enormous scale worldwide. The tissue culture technique, also known as micropropagation, is the sole alternative method that may be used to multiply a variety in its original form

rapidly. Micropropagation is a highly efficient method for rejuvenating or reviving well-adapted and promising local cultivars experiencing a slight decrease or degeneration in yield and vigor. This is accomplished by liberating these cultivars from illnesses caused by the buildup of viruses and other systemic pathogens during continuous vegetative culture. In addition, the effectiveness of the moist hot air treatment against the mosaic virus has not been shown. Due to the fact that the meristematic tissue maintains its immunity to viral illness, the meristem culture is the only approach that can successfully eliminate the sugarcane mosaic virus (SCMV). The cells that make up the meristem are very stable from a genetic standpoint; hence, the plants formed from them are mainly similar to those used as donors, except for the development of uncommon mutations (Walkey 1980).

The genotype may be maintained in culture for an extended time, thus providing an alternate method of preserving the sugarcane germplasm. In many countries, the generation of sugarcane seeds for commercial purposes is mainly accomplished via the use of conventional tissue culture. When all of these benefits are considered, it becomes clear that micropropagation significantly contributes to the "Seed Production Chain" in sugarcane. There has been a significant advancement in the manufacture of high-quality sugarcane seeds thanks to the development of tissue culture technology, which allows for the quick multiplication of disease-free gardening materials. Several countries in the Asia-Pacific area, including Australia, India, and the Philippines, have already used this technique for the purpose of commercial seed production. The advantages of this technology have been apparent via the quick multiplication and dissemination of elite varieties, as well as through the enhanced output of sugarcane. Within a short amount of time, the tissue culture method, also known as micropropagation, may produce disease-free, uniform, and large plantlets. On the contrary, the high cost of micropropagation is a significant bottleneck that prevents its widespread use in sugarcane. According to Furtado et al. (2011), some technologies may reduce the costs associated with sugarcane micropropagation. These technologies include directly regenerating entire plantlets on the same medium and substituting ex-vitro rooting for in vitro rooting in standard micropropagation protocols. It has been noted that the present cost of manufacturing is the most significant constraint of the technology. The labor necessary for the transfer of the cultures takes place on a two-week cycle, accounting for most of the budgetary expenditure. The cost may be reduced in the future by using process automation or semi-automation (Krishna and Varma 2022).

25.6.2 Temporary Immersion Bioreactor System (TIBS) for Sugarcane Micropropagation

This novel bioreactor system for micropropagation has a number of features that have been specially intended to simplify its operation and minimize expenses within the manufacturing process. The TIBS is based on the idea of momentary immersion

as its operating premise. It is made up of two vessels, one of which is filled with plant tissues, and the other of which is filled with liquid media. These two vessels are connected to one another using an autoclavable silicon tube. Since the whole system is sealed off and automated, it provides the optimal conditions for effective micropropagation on a wide scale (Bordonal et al. 2018). TIBS has the benefit of minimizing the amount of physical labor required; it allows for the changing of culture medium rather than the transfer of plants; it does not need the use of jellifying agents; and it makes nutrients more easily accessible to culture tissues. The most significant difficulty associated with this technology is the fact that it is more challenging to manage microbial contamination. The TIBS is 1 in its operational state in our biofactory setup. A TIBS technique that is both effective and efficient has been devised and tested using sugarcane types that sugar estates desire. The meristem tip or immature leaf whorls are first used to begin the microshoots of the individual variety. Following this, the microshoots are gradually transferred to medium with lower quantities of gelling agent weekly to complete the conditioning process for liquid media conditions. As a consequence of this, the quantity of microshoots of the variety has increased at a faster rate (Usman 2015).

25.6.3 Mudas Pre-Brotadas (MPB) or Pre-germinated Seedling Technique

The Mudas Pre-Brotadas The Cana Program of Brazil has created a new technology known as the pre-germinated sugarcane seedlings nursery (MPB). The purpose of this technology is to improve the efficiency and economic benefits associated with the development of nurseries, the replanting of areas that are sold, and maybe the rehabilitation and extension of areas that are used for sugarcane fields. Moreover, the decrease in the quantity of seedlings that are introduced into the field is an additional significant advantage. According to Segalo (2014), the nursery for pre-germinated sugarcane seedlings is also known as the MPB. In some instances, the seed cutter is used to manually cut the seed canes into single-budded setts; whereas in other cases, the hacksaw is involved. The individual bud sets are then brushed with a gentle detergent solution and rinsed under running water. The sets are immersed in a fungicide solution containing 2% for two (2) hours, stirring them periodically. After that, the sets are washed with water and completely submerged in a 2% clorox solution, a commercial bleach, for either 0 minutes, 15 minutes, or 30 minutes. The treatment that included no clorox was used as the control. In the next step, the setts were washed in water three (3) times before being planted in germination trays with either bagassecilo or coconut husk serving as the substrate (Ma et al. 2014).

25.7 Thermotherapy for Disease Eradication

Since this increased output has been accomplished by expanding the planted area rather than productivity, which has remained the same since 2005/06, when it was reported at 74.3 ton/ha, the sugarcane crop is being scrutinized. Over ten years later, during the 2019/20 crop season, it was only marginally better, hitting 76.1 ton/ha (CONAB, 2020). This occurs throughout the crop season. Ratoon stunt (RSD), which is produced by *Leifsonia xyli* subsp. xyli (Lxx), is anticipated to significantly impact sugarcane production even though numerous variables influence sugarcane output (Snyman et al. 2011). According to Solomon (2016), the second method involves selecting healthy stalks from commercial fields at the plant cane or first ratoon. These stalks are then subjected to a laboratory diagnostic test, subjected to heat treatment, and used to establish nurseries for seed cane production. A total of 9.2% of the total consisted of plantings that used MPB seed cane, while 90% of the total included plantings that utilized sugarcane stalks. The remaining 0.3% depended on the planting material that was developed by tissue culture (Evans et al. 1984).

Since RSD does not exhibit any hallmark symptoms, laboratory-based diagnosis is essential for identifying Lxx. Various techniques are available, including microscopy, serological, and DNA-based approaches, which may be used for this purpose. The method used by the Brazilian business differentiates itself according to the origin of the seed cane. Since fewer mother plants need to be examined, the PCR (Polymerase Chain Reaction)-based diagnostic test is mainly used to certify MPB seed canes. This makes the test more cost-effective for businesses. In contrast, the serology technology continues to be the preferred method for indexing the mechanical billet planting material. This is due to the fact that it allows for the testing of a substantial number of samples per field in a way that is both cost-effective and efficient (Feldman et al. 1989).

In Brazil, two different combinations of thermotherapy are utilized: a hot water treatment (HWT) at 50 ° C for two hours and 52 ° C for 30 min (de Morais et al. 2015). Serology, quantitative PCR (qPCR), and nested PCR were all methods that were used to detect Lxx following treatment with HWT at a temperature of 52 °C for thirty minutes. However, the findings of these methods were inconsistent. However, Lxx was not found following the same HWT, and this was the case regardless of whether the analysis was performed using standard PCR techniques or microscopically. In addition, the 50 °C for 2 h HWT yielded contradictory findings, with some research claiming that it eliminated all pathogens (Strachan et al. 2022).

Among the parameters that influence the control of RSD via the application of HWT are cultivar features, diagnostic procedures used to evaluate its effectiveness, HWT conditions, and the initial Lxx titers of sugarcane setts. On the contrary, integrated information cannot be deduced from this research since they did not simultaneously analyze several different parameters. To improve the efficiency of the exclusion procedures, it is essential to get clarification on the precise impact that each of these components alone and collectively make.

25.8 Molecular Diagnostics for Pathogen Detection

Conventional plant pathogen diagnostics typically involve the utilization of one or more of the following techniques: pathogens that can be seen with the naked eye, methods for isolating and cultivating them, and morphological approaches based on culture. These methods involve the detection of whole organisms or DNA proteins associated with pathogens. These procedures are generally reliable and have been around for a long time, but they are also rather pricey and are often carried out in a centralized laboratory. As a result, they are not suited for diagnostic testing that is straightforward, quick, and economical in on-farm applications (Tiwari et al. 2012).

Several effective on-site diagnostic instruments have recently been created to precisely and promptly identify and measure plant diseases. These on-site diagnostics have the potential to be applied in the field as quick first-pass presence/absence screening techniques. Additionally, they may be exploited in the early phases of pathogen establishment and disease progression, far before significant economic harm is incurred. It is important to note that these tests make it possible to diagnose accurately in areas previously thought to be free of pathogens. As a result, they provide early warnings, which allows for implementing preventative management measures (Cursi et al. 2022).

There are often two primary integrated components that make up portable diagnostic equipment. One of these components is responsible for sample preparation, and the other is responsible for detection. Multidisciplinary expertise is often required for this integration. Some examples of such expertise include microbiology.

25.9 Sugarcane Production-Related Impacts

Increasing global sustainability while simultaneously satisfying the need for energy has been suggested as a potential option by cultivating bioenergy crops (Verma et al. 2022a, b). Several studies have brought attention to the environmental advantages of sugarcane ethanol as a viable alternative for reducing greenhouse gas emissions compared to other biofuel feedstocks. These studies provide a summary of the generally examined criteria (such as energy balance, greenhouse gas savings, biofuel production, and water footprint) and compare the primary energy crops (such as maize, sugarcane, wheat, sugar beet, and sorghum) that are used to generate bioethanol throughout the globe. These findings are based on research that was conducted on life cycle assessment (Andreato et al. 2022).

According to reports, ethanol derived from sugarcane is the most efficient choice for all of the factors that have been discussed. It has the greatest energy balance (9.1) when compared to maize (1.4), wheat (5.2), sugar beet (2.0), and sorghum (2.8). Therefore, the replacement of fossil fuels with ethanol derived from sugarcane results in a reduction in greenhouse gas emissions by 85 percent. This is in contrast to the emission avoidance of 30 percent for maize, 45 percent for sugar

beets, 53 percent for sorghum, and 64 percent for wheat. During the industrial phase, the use of biomass as a biofuel for power production is the primary component that contributes to the achievement of the targeted reductions in emissions. In comparison to the average yield of sugarcane-based ethanol (L ha^{-1}), which is 5250, the yield of sugar beets is 5250, the yield of maize is 4010, the production of sorghum is 2990, and the yield of wheat is 2450. Sugarbeet has the lowest water footprint among ethanol-producing crops, making it more efficient (Hendre et al. 1975).

25.9.1 Green Mechanized Harvesting and Tillage Practices

Burning residues before harvest has considerable negative consequences on human health. This is in addition to the discharge of soot, greenhouse gases, and particulate matter into the atmosphere. According to several studies, there is a link between the emissions of particulate matter from the burning of sugarcane and the occurrence of illnesses, such as asthma, respiratory disorders, lung cancer, and hypertension hospital admissions. The burning of sugarcane results in an emission of 941 kilograms of carbon dioxide equivalent per hectare per year. This amount accounts for 30.3% of the overall greenhouse gas emissions associated with sugarcane cultivation compared to other sectors. Over the last decade, a progressive decline from 61 to 6% (2.3 million hectares) in areas under sugarcane burning in south-central Brazil has been seen. This decrease has been particularly noticeable in São Paulo, where legislation has been implemented to prohibit sugarcane burning. Similarly, a change in harvest management improved more than 90 percent in terms of human health consequences (Fig. 25.2). Furthermore, it is anticipated that the potential for global warming and black carbon emissions will be reduced by 70 percent and 216 times, respectively, in the future with total automation (Kaur and Kapoor 2016).

Due to its advantages in terms of agronomic and environmental considerations, non-burning harvesting is well recognized in the scientific literature as a practice that benefits all parties involved. On the contrary, in the green harvest system, the greater levels of soil compaction have been acknowledged as the primary problem in traditional sugarcane farming systems in Brazil. This is due to the heavy and intensive traffic that occurs during mechanical harvest and transport. It has been noted that there has been a significant decrease in the amount of sugarcane harvested since 2008 during the automation of activities, such as harvesting and planting. Nevertheless, the high degree of harvest automation leads to a rise in fuel use, which in turn contributes to increases in greenhouse gas emissions overall. According to Lal et al. (2015), the Brazilian sugarcane industry significantly contributed to greenhouse gas emissions, accounting for around forty percent of the total emissions in 2009. This was mostly due to the high use of diesel in the automated harvesting process. The use of renewable fuels, such as biodiesel derived from vegetable oils and fuel ethanol, as well as the enhancement of the efficiency of equipment and transportation vehicles are among the potential alternatives that have

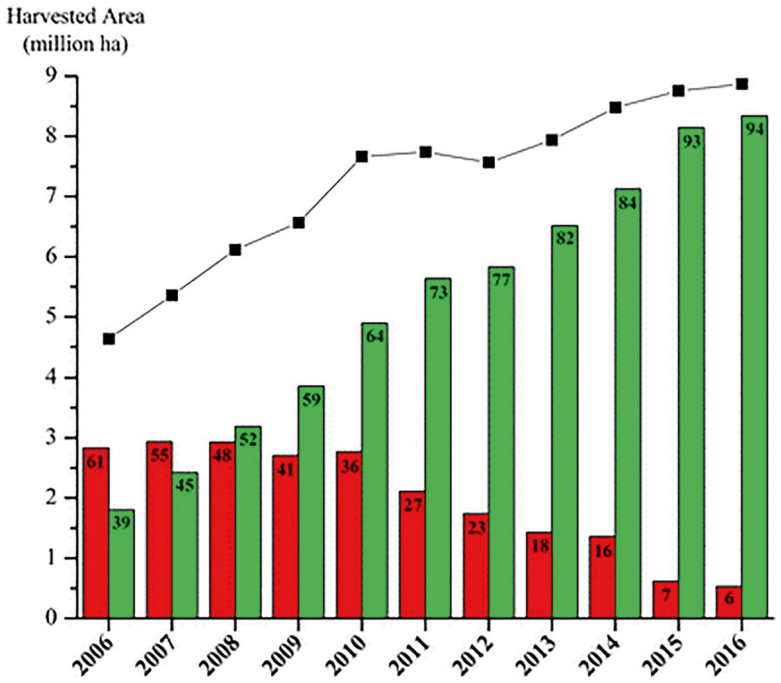

Fig. 25.2 Production rate of sugarcane through green technology

the potential to decrease the greenhouse gas emissions that are linked with diesel (Sengar et al. 2011).

25.9.2 Commercialization of Clean Seed Production

The methods of tissue culture are now becoming more significant instruments for the development of crops and the fast replication of chosen clones. Using these strategies in breeding, propagation, disease removal, generation of clones adapted for abiotic and biotic conditions, conservation of genetic resources, and other areas has garnered significant attention. The process of in vitro micropropagation is now receiving a lot of interest for its ability to successfully multiply novel kinds on a large scale in a relatively shorter amount of time in several different crops, including sugarcane. However, applying the micropropagation technique in sugarcane has several benefits, including the rapid multiplication of newly released varieties and the revitalization of old varieties that have deteriorated. A number of issues have been remedied to a significant degree, as mentioned in this evaluation, but specific concerns still remain. However, certain limitations still need to be handled via extensive study efforts for the sugarcane seed production program. This is necessary

to guarantee that the use of this approach will be both safe and effective (Varma et al. 2022).

25.9.3 Microbial Contamination

Culture contamination is a severe issue that decreases the frequency of shoot culture initiation from the source explants and reduces the total number of shoots generated at different cycles, owing to the loss of cultures. This is a direct result of the contamination of cultures. The elimination of all microbes from plant tissue cultures has been a goal that researchers have pursued on several occasions but with little to no success. Even in an environment that seems to be clean, it is possible that it will not be possible to get genuinely axenic cultures. This is consistent with the findings of specific investigations. Surprisingly, the presence of microorganisms has been shown to improve the cultures, promote organogenesis, regenerate more robust plantlets, improve root systems, and enhance transplant tolerance (Sharma et al. 2021, 2022).

The most prevalent pollutants found in sugarcane cultures are microorganisms that belong to the classes of bacteria known as saprophytes, such as Enterobacter and Bacillus species; capturing microbial spores, owing to their hairy surface, is the source of the acute contamination that is produced by fungal species. This contamination happens at the moment when the culture is initiated. Furthermore, it manifests itself during the process of separating. It is possible for these pollutants to move from one bottle to another, which may occasionally lead to the loss of a significant number of cultures. Since the surface sterilization does not affect the endogenous systemic pollutants, it is exceedingly difficult to maintain control over them (Carvalho et al. 2017; Wu et al. 2022; Bhatt et al. 2024).

Some antibiotics have been shown to be unsuccessful in limiting the contamination. In contrast, others have been found to have a phytotoxic impact, suggesting that they are unsuitable for integration into the medium. The side effects of these substances on branch morphogenesis, multiplication ratio, and other aspects of plant development need to be investigated further to identify whether or not these properties are passed on to subsequent generations. In recent years, chemical sterilants, such as sodium hydroxide and mercury chloride, typically used for the surface sterilization of explants, have been included in the medium at low concentrations for media sterilization. Some producers of reagents are now promoting antimicrobial supplements. These supplements may be added to the medium while the subculture is being performed (Viswanathan and Rao 2011).

25.9.4 Phenolics Excretion

"When sugarcane is first cultured, the young meristematic tissues contain significant concentrations of phenolic compounds, which are discharged in large quantities into the medium. Under in vitro conditions, found that the excretion of phenolics seems to be connected with the stressors that are applied due to excision, wounds, growth regulators, and environmental changes. Following the process of secretion, these phenolics undergo oxidation, forming black brown oxidation products, which in turn causes the medium to become darker. Phenolics in their oxidized state are responsible for the death of explants, most likely via the process of promoting tissue oxidation. The vitrified sugarcane shoots grown in tissue cultures have a glassy look, become thick, and soak up water. Additionally, the leaves of these shoots become transparent. It is often challenging to root vitrified shoots even though they continue to develop and multiply satisfactorily. This phenomenon is usually related with the medium. This method may significantly slow down the pace of shoot regeneration (Suprasanna et al. 2011).

25.9.5 Somaclonal Variation

Based on the findings, sugarcane cultures exhibit a significant diversity from cell to cell and within differentiated plantlets. This variation, created via tissue culture and referred to as "somaclonal variation,"primarily depends on the many pathways involved in plant regeneration. It is possible to regenerate sugarcane plants in vitro through three distinct processes. To ensure that the genetic integrity of the donor material is maintained in the micropropagated offspring, the goal of micropropagation is to make clones that are identical to the parent plant. When it comes to crops like sugarcane, where clones with a morphological characteristic that has been changed are transformed into a distinct variety, this matter is of great significance. The formation of axillary shoots, which is the first route outlined above, has been regarded as the most reliable and favored micropropagation technique to preserve clonal integrity (Hanlon et al. 2000). This results in plants that are essentially similar to those produced by the previous cycle. Nevertheless, the differentiation between the various regeneration routes becomes less clear during later cycles of multiplication, which are characterized by intense multiplication conditions. Random damage can shoot cultures to give birth to deformed structures during splitting and subculture. Several researchers have established the ability to induce direct somatic embryogenesis via inflorescence segments. They also noted that this technology has the potential to be effective in the quick multiplication of elite sugarcane varieties as well as in the development of transgenics that do not include any somaclonal mutation. The direct somatic embryogenesis (DSEM) technique was particularly helpful for large-scale clonal replication, which was both cost-effective and efficient. Additionally, it offered a novel target explant source for genetic

transformation. The management of a culture in such a manner that just one of the pathways of shoot regeneration is followed has not been achievable in commercial practice up to this point, despite the fact that each of the pathways of shoot regeneration outlined above reflects a distinctive differentiation process. The intended route may predominate when appropriate cultural methods are used with proper explants (Li et al. 2016).

To get a better understanding of the manner and extent of differentiation, it is necessary to do more research on the materials that are increasing via the use of electron microscopy and histology. The information acquired using this method may be useful in managing the shoot regeneration that occurs along channels that are not wanted. It is also important to use molecular tools to determine whether or not the plants that have been micropropagated are genetically uniform. Among the characteristics linked with crops are the intrinsic variations present in the original plant material. These variations include chromosomal mosaicism, chimerism, aneuploidy, and polyploidy. It is common for genetically complex crops, such as sugarcane, to be affected by these variables (Bordonal et al. 2018).

25.9.6 Higher Cost of Production

Creating a high-quality and consistent planting material that can be replicated throughout the year is the primary benefit of tissue culture technology. This material may be used to cultivate plants throughout the year. However, the technique requires a significant amount of energy, work, and cash to operate. Despite the fact that labor is very inexpensive in many developing nations, there is often a shortage of resources including skilled staff. In addition, the cost of things like power and clean water is high (Heinz et al. 1969).

25.10 Strategies for Successful Seed Production Program

Upon transplanting, the tissue-grown plantlets produced a very high number of tillers and millable canes; nevertheless, the cane stalks are typically thin because of the large drop in the cane diameter. It also seems that the canes have been derived from the donor since the stalks are so slender. There is a possibility that plantlets will be damaged during transit, and there is also a significant occurrence of plant death after transplanting. These limits provide a negative message to the cane farmers. As a result, it is difficult to convince them to embrace tissue grown plantlets as the seed material instead of traditional plantlets. In light of this, implementing a three-tiered method of seed multiplication has been proposed, similar to the one now being used in Uttar Pradesh as part of the traditional seed multiplication program (Viswanathan 2021).

Through the three-tier system, the plantlets that have been grown via tissue culture should be used to cultivate breeder seed nurseries at institutional farms under the close supervision of scientists. In later years, the breeder seed cane that was acquired in this manner needed to be used to cultivate Sugar Tech 123 foundation and certified seed nurseries via standard set planting techniques. An expert group of scientists consisting of a breeder, a pathologist, and an entomologist must be responsible for the rigorous monitoring and certification of every nursery. Foundation and accredited seed nurseries have a modest rise in the cane diameter, which eventually returns to its usual level. The certified seed that is generated in this manner ought to be transferred to the cane farmers for the purpose of general planting (Fahmy et al. 2017).

25.11 Conclusion

When it comes to the quick multiplication of high-value decorative plants, such as orchids, which are propagated slowly by vegetative methods, the in vitro micropropagation techniques seem to be practical. This is because the preservation of genetic stability in the subclones is of less relevance. The fact that the in vitro approach was successfully used in the micropropagation of one crop does not necessarily mean that it applies to all other crops' cultivation. The sole approach that is considered to be adequate for directly assessing the uniformity of micropropagated sugarcane plants is their field assessment for different sugar and yield contributing characteristics. This evaluation is carried out by comparing the specimens to an equivalent population of plants that have been grown traditionally. There is a possibility that variations, if any, might provide a chance for clonal development via selection (Jalaja et al. 2006), while genuine clones could be used to cultivate seed nurseries using traditional practice. Suppose the phenotypic variation is discovered to be more than the acceptable limit. In that case, all materials will be rejected, resulting in the unnecessary expenditure of time and energy.

Because of this, it is recommended that plantlets grown in vitro should not be immediately sent to cane producers for general culture without first ensuring that they are genetically consistent. Instead, the plants that have been micropropagated need to be subjected to the three-tier seed multiplication procedure. Cane seed acquired in this manner is genetically consistent and approved and may be used for general planting. Not only will this approach reduce the cost of tissue culture planting material, but it will also increase the use of this practice among farmers. In conclusion, tissue culture methods have the potential to be exploited more effectively for the purpose of fast multiplication of sugarcane, provided those certain restrictions, such as the high cost of production and the somaclonal variance, are addressed and resolved.

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