Research Insight Open Access

Advancements in Symbiotic Nitrogen Fixation: Enhancing Sugarcane Production

Wenzhong Huang
Tropical Plant Research Center, Hainan Intitutes of Tropical Agricultural Resources, Sanya, 572025, Hainan, China
Corresponding email: wenzhong.huang@hitar.org
Molecular Soil Biology, 2024, Vol.15, No.1 doi: 10.5376/msb.2024.15.0004
Received: 02 Jan., 2024
Accepted: 03 Feb., 2024
Published: 14 Feb., 2024

Preferred citation for this article:
Huang W.Z., 2024, Advancements in symbiotic nitrogen fixation: enhancing sugarcane production, Molecular Soil Biology, 15(1): 28-36 (doi: 10.5376/msb.2024.15.0004)

Abstract As an important crop worldwide, sugarcane requires sustainable agricultural practices to meet the growing demand. This study explores the latest progress in symbiotic nitrogen fixation as a sustainable alternative to traditional fertilizers. Symbiotic nitrogen fixation involves beneficial bacteria converting atmospheric nitrogen into a form that can be utilized by plants, thereby improving soil health and reducing dependence on fertilizers. The study discussed the biological mechanisms and gene interactions of this process in sugarcane, emphasizing the latest technological innovations including genetic engineering and biological fertilizers. Detailed case studies demonstrate the practical benefits of these technologies, including increased yield and improved soil health. Despite challenges such as biological, environmental, and socio-economic barriers, the potential for optimizing nitrogen fixation through genetic modification and precision agriculture is promising. Supportive policies are crucial for the widespread adoption of these technologies. This study emphasizes the crucial role of symbiotic nitrogen fixation in improving sugarcane productivity and sustainability, and calls for continued research and development in this field.
Keywords Sugarcane (*Saccharum officinarum* L.); Symbiotic nitrogen fixation; Sugarcane production; Technological progress; Case studies; Challenges and opportunities

Sugarcane (*Saccharum officinarum* L.) is a major crop cultivated in tropical and subtropical regions around the world. It is a primary source of sugar and bioethanol, contributing significantly to the economies of many countries, particularly in Asia, South America, and Africa. The global demand for sugarcane is driven by its use in food products, biofuels, and various industrial applications. However, the cultivation of sugarcane is highly dependent on the use of nitrogen fertilizers, which are costly and have detrimental environmental impacts, including soil degradation and water pollution (Lincoln and Vitousek, 2016; Guo et al., 2020; Singh et al., 2021).

Nitrogen fixation is a critical process in agriculture, where atmospheric nitrogen (N_2) is converted into ammonia (NH_3), a form that plants can utilize. This process is primarily carried out by diazotrophic bacteria, which can either live freely in the soil or form symbiotic relationships with plants. Symbiotic nitrogen fixation, particularly in legumes, has been extensively studied and utilized to reduce the need for synthetic nitrogen fertilizers. However, extending this capability to non-leguminous crops like sugarcane presents a significant opportunity to enhance sustainable agricultural practices (Graham and Vance, 2000; Biswas and Gresshoff, 2014; Mus et al., 2016).

The primary objective of this study is to explore advancements in symbiotic nitrogen fixation to enhance sugarcane production. This involves investigating the potential of various diazotrophic bacteria to form effective symbiotic relationships with sugarcane, thereby reducing the reliance on synthetic nitrogen fertilizers. The study aims to identify and characterize bacterial strains that can promote nitrogen fixation and improve sugarcane growth under different environmental conditions. By leveraging genetic and molecular insights, the study seeks to develop biofertilizers that can enhance nitrogen use efficiency and contribute to sustainable sugarcane cultivation (Martins et al., 2020; Singh et al., 2021; Luo wt al., 2023). The expected outcomes include increased sugarcane yield, reduced environmental impact, and a deeper understanding of the mechanisms driving symbiotic nitrogen fixation in non-leguminous crops.

1 Symbiotic Nitrogen Fixation: An Overview

1.1 Definition and mechanisms of symbiotic nitrogen fixation

Symbiotic nitrogen fixation (SNF) is a biological process where certain microorganisms convert atmospheric nitrogen (N_2) into ammonia (NH_3), a form that plants can readily assimilate. This process is facilitated by the enzyme nitrogenase, which is encoded by the *nifH* gene, among others. The symbiotic relationship typically involves bacteria colonizing plant roots or tissues, forming specialized structures such as nodules where nitrogen fixation occurs. This mutualistic interaction allows plants to access a vital nutrient without relying on synthetic fertilizers, thereby promoting sustainable agricultural practices (Guo et al., 2020; Singh et al., 2021).

1.2 Key organisms involved

Several key organisms are involved in SNF, including rhizobia, endophytic bacteria, and diazotrophs. Rhizobia are well-known for their symbiotic relationship with leguminous plants, forming root nodules where nitrogen fixation takes place. Endophytic bacteria, such as *Enterobacter roggenkampii* and *Gluconacetobacter diazotrophicus*, reside within plant tissues and contribute to nitrogen fixation without forming nodules (Guo et al., 2020; Saranraj et al., 2021; Luo et al., 2023). Diazotrophic bacteria like *Pantoea dispersa* and *Enterobacter asburiae* have also been identified as significant contributors to nitrogen fixation in sugarcane, enhancing plant growth and stress tolerance (Singh et al., 2021).

1.3 Benefits of symbiotic nitrogen fixation in crop production

The benefits of SNF in crop production are manifold. Firstly, it reduces the dependency on synthetic nitrogen fertilizers, which are costly and environmentally damaging due to their contribution to soil and water pollution (Guo et al., 2020; Singh et al., 2021). Secondly, SNF enhances plant growth and yield by providing a consistent and sustainable nitrogen source. For instance, studies have shown that nitrogen-fixing bacteria like *Pantoea dispersa* and *Enterobacter asburiae* can significantly improve sugarcane physiological parameters such as plant height, shoot weight, and chlorophyll content (Singh et al., 2021). Additionally, SNF contributes to better disease management and stress tolerance in crops, as evidenced by the increased expression of stress-related genes in sugarcane varieties colonized by nitrogen-fixing bacteria (Guo et al., 2020; Singh et al., 2021).

2 Current Practices in Sugarcane Cultivation

2.1 Conventional methods of nitrogen fertilization in sugarcane farming

The traditional approach to sugarcane cultivation involves the extensive use of synthetic nitrogen fertilizers to meet the high nitrogen demands of the crop. These fertilizers are produced from atmospheric nitrogen and natural gas, a process that has been scaled up significantly since the Green Revolution to boost crop yields (Pankievicz et al., 2019). However, the efficiency of nitrogen use in sugarcane is relatively low, leading to substantial nitrogen losses and necessitating frequent applications (Luo et al., 2023).

2.2 Environmental and economic challenges associated with chemical fertilizers

The reliance on synthetic nitrogen fertilizers poses several challenges. Environmentally, the excessive use of these fertilizers contributes to greenhouse gas emissions and soil degradation, impacting the sustainability of agricultural practices (Luo et al., 2023). Economically, the production and application of nitrogen fertilizers represent a significant expense for farmers, particularly in developing countries where the cost can be prohibitive (Mus et al., 2016; Junior et al., 2020). These challenges underscore the need for more sustainable and cost-effective nitrogen management strategies.

2.3 Need for sustainable alternatives

Given the environmental and economic drawbacks of synthetic nitrogen fertilizers, there is a pressing need for sustainable alternatives. Biological nitrogen fixation (BNF) presents a promising solution, as it involves the natural conversion of atmospheric nitrogen into a form that plants can use, facilitated by symbiotic relationships between plants and nitrogen-fixing bacteria (Mus et al., 2016; Pankievicz et al., 2019). Recent research has focused on extending these symbiotic relationships to non-leguminous crops, including sugarcane, to reduce dependency on chemical fertilizers and enhance nitrogen use efficiency (Pankievicz et al., 2019; Luo et al., 2023).

By leveraging the genetic diversity and high BNF capacity of wild sugarcane progenitors like *Saccharum spontaneum* L., it is possible to develop new sugarcane varieties that are more resilient and efficient in nitrogen utilization (Luo et al., 2023).

3 Mechanisms of Symbiotic Nitrogen Fixation in Sugarcane

3.1 Biological pathways and interactions between sugarcane and nitrogen-fixing bacteria

The interaction between sugarcane and nitrogen-fixing bacteria involves complex biological pathways. Diazotrophic bacteria, such as *Pseudomonas*, *Bacillus*, and *Enterobacter* species, colonize the rhizosphere and endophytic tissues of sugarcane, facilitating nitrogen fixation (Singh et al., 2020). These bacteria possess the *nifH* gene, which encodes for the nitrogenase enzyme responsible for the conversion of atmospheric nitrogen to ammonia (Singh et al., 2021). The colonization of sugarcane roots by these bacteria leads to the formation of specialized structures and physiological modifications that enhance nitrogen uptake and assimilation (Lindström and Mousavi, 2019; Aasfar et al., 2021).

3.2 Genetic and molecular aspects of nitrogen fixation in sugarcane

The genetic and molecular mechanisms underlying nitrogen fixation in sugarcane involve the regulation of various genes and metabolic pathways. Studies have shown that the expression of genes related to nitrogen metabolism, such as *nifH*, and stress-related genes, including those encoding for catalase, superoxide dismutase, and phenylalanine ammonia-lyase, are upregulated in sugarcane inoculated with nitrogen-fixing bacteria (Singh et al., 2020). Additionally, transcriptome analyses have revealed differential gene expression in sugarcane genotypes with varying BNF efficiencies, highlighting the role of auxin signaling and microorganism perception pathways in modulating nitrogen fixation (Carvalho et al., 2022; Luo et al., 2023).

3.3 Recent research findings on enhancing nitrogen fixation efficiency

Recent research has focused on enhancing the efficiency of nitrogen fixation in sugarcane through the selection and inoculation of effective diazotrophic strains. For instance, the inoculation of sugarcane with *Pseudomonas koreensis* CY4 and *Pseudomonas entomophila* CN11 has been shown to significantly increase nitrogen fixation and plant growth under greenhouse conditions (Singh et al., 2023). Similarly, the use of *Bacillus megaterium* CY_5 and *Bacillus mycoides* CA_1 strains has demonstrated substantial improvements in nitrogen accumulation and pathogen resistance in sugarcane (Figure 1) (Singh et al., 2020). Furthermore, the identification of high-BNF sugarcane genotypes and the exploration of their genetic diversity offer promising avenues for developing nitrogen-efficient sugarcane varieties (Carvalho et al., 2022; Luo et al., 2023).

Singh et al. (2020) displays fluorescence (GFP) micrographs of sugarcane plant parts (leaf, stem, and root) colonized by GFP-tagged *Bacillus megaterium* (CY5) and *Bacillus mycoides* (CA1), highlighting the interaction between the bacterial strains and the plant tissues. Control plant parts (A-C) show no bacterial colonization, while the inoculated plant parts (D-I) exhibit green fluorescence indicating the presence of GFP-tagged bacteria. The images reveal bacterial colonization on the surface of roots, junction areas, stems, and leaves, with blue arrowheads marking individual or clusters of bacterial cells. This study demonstrates the successful colonization of sugarcane by beneficial bacteria, potentially enhancing nutrient uptake and plant growth. The use of GFP tagging and confocal laser scanning microscopy effectively visualizes bacterial distribution within the plant, providing valuable insights into the symbiotic relationship and its implications for sustainable agriculture. This research underlines the potential of biofertilizers in reducing chemical fertilizer dependency and promoting healthier crop growth.

4 Technological Advancements in Symbiotic Nitrogen Fixation

4.1 Genetic engineering and biotechnology approaches

Genetic engineering and biotechnology have significantly advanced our understanding and application of SNF in non-leguminous crops like sugarcane. Researchers have identified and manipulated key genes involved in nitrogen fixation, such as the *nifH* gene, which encodes a component of the nitrogenase enzyme complex responsible for converting atmospheric nitrogen into a bioavailable form (Guo et al., 2020; Singh et al., 2022). For instance, the complete genome sequencing of nitrogen-fixing bacteria like *Enterobacter roggenkampii* has revealed numerous genes associated with nitrogen metabolism, stress tolerance, and plant growth promotion, providing a blueprint for developing genetically engineered strains with enhanced SNF capabilities (Guo et al., 2020). Additionally, synthetic biology tools have been employed to optimize nitrogen-fixing microbes for better colonization and nitrogen fixation in fertilized fields, demonstrating significant agronomic benefits (Wen et al., 2021).

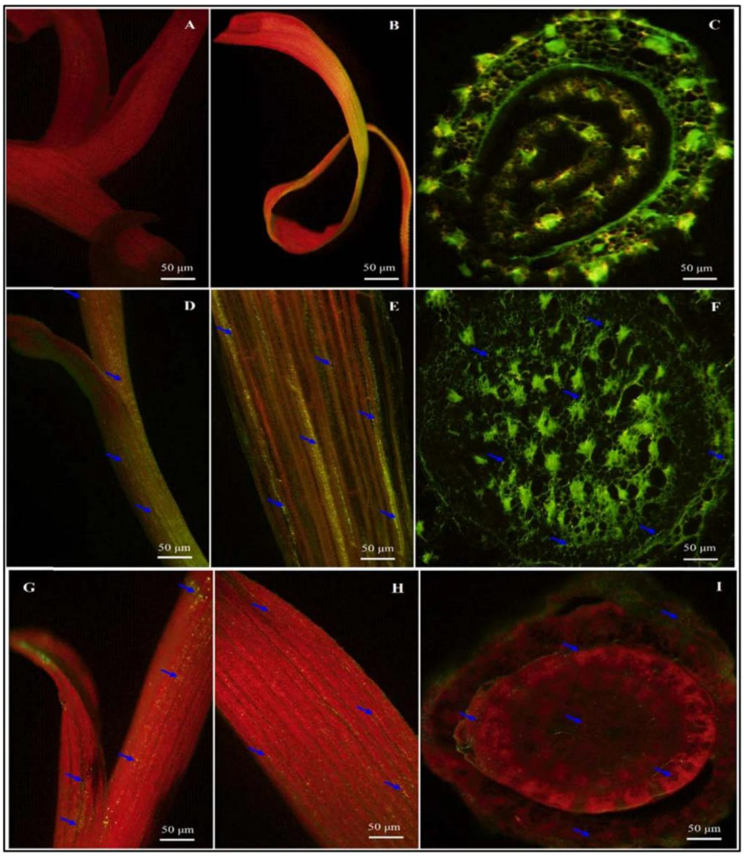

Figure 1 Fluorescence (GFP) micrographs of sugarcane plant colonized by GFP-tagged *Bacillus megaterium* (CY5) and *Bacillus mycoides* (CA1) leaf, stem, and the root of micropropagated plantlets of sugarcane variety GT11 (Adopted from Singh et al., 2020)
Image caption: Confocal laser scanning microscopic images present bacterial in green and red dots of auto-fluorescence in everywhere of plant parts respectively. (A-C) Control sugarcane plant parts and (D-I) Sugarcane plant parts inoculated with GFP tagged bacterial strains at 500-530 nm. On the surface of roots, junction area, around the whole root, stem, and leaf. Bacterial cells are indicated with blue arrowheads in single or clustered of bacteria. D-F represent CY5, and G-I represents CA1 strain with bar 50 μm (Adopted from Singh et al., 2020)

4.2 Development of nitrogen-fixing biofertilizers

The development of biofertilizers containing nitrogen-fixing bacteria is a crucial step towards reducing reliance on synthetic fertilizers. Various diazotrophic bacteria, including *Pseudomonas*, *Bacillus*, and *Enterobacter* species, have been identified and tested for their ability to fix nitrogen and promote sugarcane growth (Table 1) (Singh et al., 2020). These biofertilizers not only enhance nitrogen availability but also improve plant health by inducing defense-related gene expression and increasing the activity of enzymes involved in stress responses (Singh et al., 2021). For example, *Pseudomonas* strains have been shown to significantly increase nitrogen fixation and defense enzyme activities in sugarcane, leading to improved growth and reduced need for chemical fertilizers (Singh et al., 2023).

4.3 Integration of advanced microbiome technologies

Advanced microbiome technologies, such as high-throughput sequencing and metagenomics, have revolutionized our understanding of the complex interactions between plants and their associated microbial communities. Studies have revealed the diversity and functional potential of endophytic and rhizospheric diazotrophs in sugarcane, highlighting their role in nitrogen fixation and plant growth promotion (Singh et al., 2022; Luo et al., 2023). By characterizing the microbial communities in different sugarcane tissues, researchers have identified key bacterial families and genera that contribute to SNF and stress resilience (Singh et al., 2022). These insights enable the development of targeted microbial inoculants and the optimization of microbial consortia for enhanced SNF and crop productivity (Luo et al., 2023).

Table 1 Effect of *P. korensis* CY4 and *P. entomophila* CN11 strains inoculation on the sugarcane growth parameters (Adopted from Singh et al., 2023)

Parameters	Days	Sugarcane varieties					
		GT11			GXB9		
		Control	CY4	CN11	Control	CY4	CN11
Chlorophyll (SPAD units)	30	14.30 ± 0.26[b]	16.78 ± 0.31[a]	15.84 ± 0.29[a]	15.41 ± 0.28[c]	17.59 ± 0.32[a]	16.69 ± 0.31[b]
Leaf area (cm^2)		375.63 ± 6.90[c]	575.36 ± 10.58[b]	624.50 ± 11.48[a]	428.91 ± 7.88[c]	614.39 ± 11.29[b]	670.67 ± 12.33[a]
Plant height (cm)		25.09 ± 0.46[c]	32.06 ± 0.30[b]	34.58 ± 0.40[a]	22.06 ± 0.41[c]	25.07 ± 0.37[b]	29.07 ± 0.35[a]
Root Weight (g)		2.70 ± 0.050[b]	2.90 ± 0.035[a]	2.85 ± 0.044[a]	1.41 ± 0.63[b]	2.42 ± 0.04[a]	2.30 ± 0.04[a]
Shoot weight (g)		15.47 ± 0.36[c]	18.83 ± 0.16[a]	17.44 ± 0.20[b]	18.85 ± 0.27[c]	29.73 ± 0.18[b]	30.83 ± 0.81[a]
Chlorophyll (SPAD units)	60	31.14 ± 0.57[c]	38.03 ± 0.70[a]	33.18 ± 0.61[b]	29.02 ± 0.66[c]	33.58 ± 0.62[b]	35.54 ± 0.60[a]
Leaf area (cm^2)		540.37 ± 13.61[b]	760.07 ± 12.13[a]	751.48 ± 13.81[a]	606.63 ± 12.99[c]	753.16 ± 13.84[b]	853.60 ± 15.69[a]
Plant height (cm)		34.16 ± 0.83[c]	46.15 ± 0.77[b]	48.08 ± 0.42[a]	37.13 ± 0.68[c]	43.15 ± 0.79[b]	48.13 ± 0.70[a]
Root weight (g)		4.11 ± 0.076[c]	6.02 ± 0.111[a]	5.91 ± 0.072[b]	3.23 ± 0.151[c]	4.62 ± 0.085[a]	4.22 ± 0.077[b]
Shoot weight (g)		51.58 ± 0.95[c]	65.23 ± 1.20[a]	59.10 ± 0.54[b]	32.59 ± 0.97[c]	57.50 ± 1.06[a]	55.65 ± 0.78[b]
Chlorophyll (SPAD units)	90	29.37 ± 0.54[c]	35.12 ± 0.65[a]	33.85 ± 0.62[b]	34.05 ± 0.63[c]	37.80 ± 0.69[b]	41.21 ± 0.76[a]
Leaf area (cm^2)		938.35 ± 17.25[c]	1270.07 ± 23.35[a]	1177.26 ± 21.64[b]	750.47 ± 13.80[c]	1276.65 ± 23.47[b]	1399.87 ± 25.73[a]
Plant height (cm)		65.23 ± 1.20[c]	78.27 ± 1.44[a]	76.23 ± 1.22[b]	54.22 ± 1.18[c]	70.24 ± 0.19[b]	72.34 ± 1.29[a]
Root weight (g)		6.28 ± 0.41[c]	9.84 ± 0.18[a]	8.43 ± 0.13[b]	5.15 ± 0.79[c]	7.49 ± 0.46[b]	8.97 ± 0.09[a]
Shoot weight (g)		86.30 ± 2.14[b]	117.50 ± 2.17[a]	118.04 ± 1.80[a]	82.59 ± 1.52[c]	138.12 ± 3.28[a]	117.41 ± 2.18[b]

4.3 Integration of advanced microbiome technologies

Advanced microbiome technologies, such as high-throughput sequencing and metagenomics, have revolutionized our understanding of the complex interactions between plants and their associated microbial communities. Studies have revealed the diversity and functional potential of endophytic and rhizospheric diazotrophs in sugarcane, highlighting their role in nitrogen fixation and plant growth promotion (Singh et al., 2022; Luo et al., 2023). By characterizing the microbial communities in different sugarcane tissues, researchers have identified key bacterial families and genera that contribute to SNF and stress resilience (Singh et al., 2022). These insights enable the development of targeted microbial inoculants and the optimization of microbial consortia for enhanced SNF and crop productivity (Luo et al., 2023).

5 Case Study

5.1 Detailed examination of a successful implementation of symbiotic nitrogen fixation in sugarcane production

The case study by Martins et al. (2020) provides a comprehensive analysis of the successful implementation of symbiotic nitrogen fixation (SNF) in sugarcane production. This study highlights the potential of SNF to enhance sugarcane yield, improve soil health, and offer economic benefits by reducing the dependency on synthetic nitrogen fertilizers.

5.2 Overview of the project: location, methodology, and key stakeholders
The project was conducted in Brazil, a leading sugarcane-producing country. The methodology involved the inoculation of sugarcane cultivars with diazotrophic bacteria, specifically targeting the enhancement of biological nitrogen fixation (BNF). The key stakeholders included agricultural researchers, local farmers, and government agricultural agencies. The field experiments were meticulously designed to compare the performance of inoculated and non-inoculated sugarcane plants over multiple growing seasons (Guo et al., 2020; Martins et al., 2020; Singh et al., 2021).

5.3 Results and impact on yield, soil health, and economic benefits
The results demonstrated a significant increase in nitrogen accumulation in the aerial tissues of inoculated sugarcane cultivars. For instance, cultivar RB867515 showed an increase from 147 to 199 kg N ha^{-1}, and cultivar RB92579 from 126 to 192 kg N ha^{-1} (Martins et al., 2020). The inoculation also led to a higher proportion of nitrogen derived from BNF, contributing to over 64% of the total nitrogen in the plants. This increase in nitrogen availability translated into improved sugarcane yields and better soil health, as evidenced by enhanced microbial activity and nutrient cycling (Guo et al., 2020; Singh et al., 2021). Economically, the reduction in synthetic nitrogen fertilizer usage resulted in lower production costs and minimized environmental pollution, offering a sustainable and cost-effective solution for sugarcane farmers (Tian et al., 2020; Saranraj et al., 2021).

5.4 Lessons learned and recommendations for future applications
The case study underscores the importance of selecting appropriate diazotrophic bacterial strains and optimizing inoculation techniques to maximize the benefits of SNF in sugarcane production. Future applications should focus on large-scale field trials to validate the findings and explore the potential of integrating SNF with other sustainable agricultural practices. Additionally, fostering collaborations between researchers, farmers, and policymakers is crucial to promote the adoption of SNF technologies and ensure their long-term success (Guo et al., 2020; Maitin et al., 2020; Saranraj et al., 2021; Singh et al., 2021).

6 Challenges and Limitations
6.1 Biological and environmental factors affecting symbiotic nitrogen fixation in sugarcane
The effectiveness of SNF in sugarcane is influenced by various biological and environmental factors. The interaction between sugarcane and nitrogen-fixing bacteria is complex and can be affected by soil pH, temperature, and salinity (Guo et al., 2020; Singh et al., 2021). For instance, certain strains of nitrogen-fixing bacteria, such as *Enterobacter roggenkampii* ED5, have shown diverse growth ranges under different stress conditions, including pH, temperature, and NaCl concentrations (Guo et al., 2020). Additionally, the presence of other microbial communities in the rhizosphere can impact the colonization and nitrogen-fixing efficiency of these bacteria (Singh et al., 2021).

6.2 Technical and infrastructural constraints
The successful implementation of SNF in sugarcane also faces technical and infrastructural challenges. The identification and isolation of effective nitrogen-fixing bacterial strains require advanced genomic and biotechnological tools (Guo et al., 2020). Moreover, the application methods for these bacteria, such as inoculation techniques, need to be optimized to ensure effective colonization and nitrogen fixation (Rosa et al., 2022). The development of biofertilizers that can be easily integrated into existing agricultural practices is essential for the practical application of SNF in sugarcane cultivation (Singh et al., 2021; Rosa et al., 2022).

6.3 Socio-economic barriers to adoption
Socio-economic factors play a crucial role in the adoption of SNF technologies in sugarcane farming. Farmers may be hesitant to adopt new practices due to the perceived risks and uncertainties associated with the use of nitrogen-fixing bacteria (Pankievicz et al., 2019). Additionally, the initial costs of implementing these technologies, including the purchase of biofertilizers and the necessary equipment, can be prohibitive for small-scale farmers (Pankievicz et al., 2019; Rosa et al., 2022). There is also a need for policy support and incentives to encourage the adoption of sustainable agricultural practices, including SNF (Pankievicz et al., 2019).

7 Future Directions and Opportunities

7.1 Potential for improving nitrogen fixation through genetic modification

Genetic modification offers a promising avenue for enhancing the nitrogen-fixing capabilities of sugarcane-associated bacteria. By identifying and manipulating key genes involved in nitrogen fixation and plant-microbe interactions, it is possible to develop bacterial strains with superior nitrogen-fixing efficiency and stress tolerance (Carvalho et al., 2022; Luo et al., 2023). For example, the overexpression of nifH and other nitrogenase-related genes could be targeted to boost the nitrogen-fixing potential of endophytic bacteria (Guo et al., 2020; Singh et al., 2022). Additionally, integrating these genetically modified bacteria into sugarcane breeding programs could lead to the development of high-BNF sugarcane varieties (Luo et al., 2023).

7.2 Role of precision agriculture and digital tools in optimizing nitrogen fixation

Precision agriculture and digital tools can play a crucial role in optimizing the application and effectiveness of nitrogen-fixing bacteria in sugarcane cultivation. Technologies such as remote sensing, soil health monitoring, and data analytics can help in the precise application of microbial inoculants, ensuring that the bacteria are delivered to the most beneficial locations within the crop system (Antunes et al., 2019; Martins et al., 2020). Furthermore, digital platforms can facilitate real-time monitoring of plant health and nitrogen levels, enabling farmers to make informed decisions about fertilizer application and microbial inoculation (Singh et al., 2023).

7.3 Policy and regulatory frameworks to support the adoption of nitrogen-fixing technologies

The successful adoption of nitrogen-fixing technologies in sugarcane cultivation requires supportive policy and regulatory frameworks. Governments and agricultural bodies need to establish guidelines and incentives for the use of microbial inoculants, ensuring their safety and efficacy (Antunes et al., 2019; López et al., 2023). Policies that promote research and development in this field, as well as the dissemination of knowledge to farmers, will be crucial in driving the widespread adoption of these sustainable practices (Carvalho et al., 2022). Additionally, international collaborations and funding initiatives can accelerate the development and implementation of nitrogen-fixing technologies in sugarcane agriculture (Martins et al., 2020).

8 Concluding Remarks

Recent research has highlighted several key advancements in the field of symbiotic nitrogen fixation for sugarcane production. Inoculation with diazotrophic bacteria has been shown to significantly increase stem yield, total dry matter, and nitrogen content in various sugarcane varieties. Indigenous nitrogen-fixing bacteria in China have demonstrated the potential to reduce nitrogen fertilization while improving sugarcane biomass and nitrogen content. Endophytic bacteria such as *Pantoea cypripedii* and *Kosakonia arachidis* have been identified as potent strains that enhance nitrogen assimilation and plant growth. Additionally, the characterization of nitrogen-fixing endophytic actinobacteria like *Streptomyces chartreusis* has provided insights into their role in promoting sugarcane growth and reducing chemical fertilizer use.

The advancements in symbiotic nitrogen fixation have significant implications for enhancing sugarcane production and sustainability. By reducing the reliance on chemical fertilizers, SNF can lower production costs and minimize environmental pollution. The use of diazotrophic bacteria as biofertilizers can improve nitrogen assimilation and plant growth, leading to higher yields and better quality sugarcane. Moreover, the adoption of SNF practices can contribute to sustainable agriculture by promoting eco-friendly and cost-effective farming methods.

The future outlook for symbiotic nitrogen fixation in sugarcane production is promising, with ongoing research likely to uncover new bacterial strains and mechanisms that further enhance nitrogen assimilation and plant growth. However, there is a need for continued research to optimize the application of diazotrophic bacteria in different soil types and environmental conditions. Additionally, understanding the interactions between sugarcane plants and diazotrophic bacteria at the molecular level can provide insights into improving the efficiency of SNF. Future studies should also focus on the long-term impacts of SNF on soil health and crop productivity to ensure the sustainability of these practices.

In conclusion, the advancements in symbiotic nitrogen fixation offer a viable solution to the challenges of nitrogen fertilization in sugarcane production. By harnessing the potential of diazotrophic bacteria, we can enhance sugarcane yield, reduce environmental impacts, and promote sustainable agricultural practices. Continued research and innovation in this field will be crucial for realizing the full benefits of SNF in sugarcane cultivation.

Acknowledgments

The BioSci Publisher thanks the two anonymous peer reviewers for their professional review of this article and for providing valuable revision suggestions.

Conflict of Interest Disclosure

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

References

Aasfar A., Bargaz A., Yaakoubi K., Hilali A., Bennis I., Zeroual Y., and Kadmiri I., 2021, Nitrogen fixing azotobacter species as potential soil biological enhancers for crop nutrition and yield stability, Frontiers in Microbiology, 12.
https://doi.org/10.3389/fmicb.2021.628379

Antunes J., Freitas A., Oliveira L., Lyra M., Fonseca M., Santos C., Oliveira J., Araújo A., and Figueiredo M., 2019, Sugarcane inoculated with endophytic diazotrophic bacteria: effects on yield, biological nitrogen fixation and industrial characteristics, Anais da Academia Brasileira de Ciencias, 91(4): e20180990.
https://doi.org/10.1590/0001-3765201920180990

Biswas B., and Gresshoff P., 2014, The role of symbiotic nitrogen fixation in sustainable production of biofuels, International Journal of Molecular Sciences, 15: 7380-7397.
https://doi.org/10.3390/ijms15057380

Carvalho T., Rosman A., Grativol C., Nogueira E., Baldani J., and Hemerly A., 2022, Sugarcane genotypes with contrasting biological nitrogen fixation efficiencies differentially modulate nitrogen metabolism, auxin signaling, and microorganism perception pathways, Plants, 11.
https://doi.org/10.3390/plants11151971

Graham P., and Vance C., 2000, Nitrogen fixation in perspective: an overview of research and extension needs, Field Crops Research, 65: 93-106.
https://doi.org/10.1016/S0378-4290(99)00080-5

Guo D., Singh R., Singh P., Li D., Sharma A., Xing Y., Song X., Yang L., and Li Y., 2020, Complete genome sequence of *Enterobacter roggenkampii* ED5, a nitrogen fixing plant growth promoting endophytic bacterium with biocontrol and stress tolerance properties, Isolated From Sugarcane Root. Frontiers in Microbiology, 11.
https://doi.org/10.3389/fmicb.2020.580081

Junior E., Lobato E., Lima B., Quadros B., Lobato A., Andrade I., and Abreu L., 2020, Advancement of nitrogen fertilization on tropical environmental, Nitrogen Fixation.
https://doi.org/10.5772/intechopen.90699

Lincoln N., and Vitousek P., 2016, Nitrogen fixation during decomposition of sugarcane (*Saccharum officinarum*) is an important contribution to nutrient supply in traditional dryland agricultural systems of Hawai'i, International Journal of Agricultural Sustainability, 14: 214-230.
https://doi.org/10.1080/14735903.2015.1071547

Lindström K., and Mousavi S., 2019, Effectiveness of nitrogen fixation in rhizobia, Microbial Biotechnology, 13: 1314-1335.
https://doi.org/10.1111/1751-7915.13517

López C., Alseekh S., Torralbo F., Rivas F., Fernie A., Amil-Ruiz F., and Alamillo J., 2023, Transcriptomic and metabolomic analysis reveals that symbiotic nitrogen fixation enhances drought resistance in common bean, Journal of Experimental Botany, 74(10): 3203-3219.
https://doi.org/10.1093/jxb/erad083

Luo T., Li C., Yan R., Huang K., Li Y., Liu X., and Lakshmanan P., 2023, Physiological and molecular insights into the resilience of biological nitrogen fixation to applied nitrogen in Saccharum spontaneum, wild progenitor of sugarcane, Frontiers in Plant Science, 13.
https://doi.org/10.3389/fpls.2022.1099701

Martins D., Reis V., Schultz N., Alves B., Urquiaga S., Pereira W., Sousa J., and Boddey R., 2020, Both the contribution of soil nitrogen and of biological N2 fixation to sugarcane can increase with the inoculation of diazotrophic bacteria, Plant and Soil, 454: 155-169.
https://doi.org/10.1007/s11104-020-04621-1

Mus F., Crook M., Garcia K., Costas A., Geddes B., Kouri E., Paramasivan P., Ryu M., Oldroyd G., Poole P., Udvardi M., Voigt C., Ané J., and Peters J., 2016, Symbiotic nitrogen fixation and the challenges to its extension to nonlegumes, Applied and Environmental Microbiology, 82: 3698-3710.
https://doi.org/10.1128/AEM.01055-16

Pankievicz V., Irving T., Maia L., and Ané J., 2019, Are we there yet? The long walk towards the development of efficient symbiotic associations between nitrogen-fixing bacteria and non-leguminous crops, BMC Biology, 17.
https://doi.org/10.1186/s12915-019-0710-0

Rosa P., Galindo F., Oliveira C., Jalal A., Mortinho E., Fernandes G., Marega E., Buzetti S., and Filho M., 2022, Inoculation with plant growth-promoting bacteria to reduce phosphate fertilization requirement and enhance technological quality and yield of sugarcane, Microorganisms, 10.
https://doi.org/10.3390/microorganisms10010192

Saranraj P., Jayaprakash A., Devi V., Al-Tawaha A., and Al-Tawaha A., 2021, Isolation and nitrogen fixing efficiency of Gluconacetobacter diazotrophicus associated with sugarcane: A review, IOP Conference Series: Earth and Environmental Science, 788.
https://doi.org/10.1088/1755-1315/788/1/012171

Singh P., Singh R., Li H., Guo D., Sharma A., Lakshmanan P., Malviya M., Song X., Solanki M., Verma K., Yang L., and Li Y., 2021, Diazotrophic bacteria *Pantoea dispersa* and *Enterobacter asburiae* promote sugarcane growth by inducing nitrogen uptake and defense-related gene expression, Frontiers in Microbiology, 11.
https://doi.org/10.3389/fmicb.2020.600417

Singh P., Singh R., Li H., Guo D., Sharma A., Verma K., Solanki M., Upadhyay S., Lakshmanan P., Yang L., and Li Y., 2023, Nitrogen fixation and phytohormone stimulation of sugarcane plant through plant growth promoting diazotrophic *Pseudomonas*, Biotechnology & Genetic Engineering Reviews, 1-21.
https://doi.org/10.1080/02648725.2023.2177814

Singh R., Singh P., Guo D., Sharma A., Li D., Li X., Verma K., Malviya M., Song X., Lakshmanan P., Yang L., and Li Y., 2021, Root-derived endophytic diazotrophic bacteria *Pantoea cypripedii* AF1 and *Kosakonia arachidis* EF1 promote nitrogen assimilation and growth in sugarcane, Frontiers in Microbiology, 12.
https://doi.org/10.3389/fmicb.2021.774707

Singh R., Singh P., Li H., Song Q., Guo D., Solanki M., Verma K., Malviya M., Song X., Lakshmanan P., Yang L., and Li Y., 2020, Diversity of nitrogen-fixing rhizobacteria associated with sugarcane: a comprehensive study of plant-microbe interactions for growth enhancement in Saccharum spp, BMC Plant Biology, 20.
https://doi.org/10.1186/s12870-020-02400-9

Singh R., Singh P., Sharma A., Guo D., Upadhyay S., Song Q., Verma K., Li D., Malviya M., Song X., Yang L., and Li Y., 2022, Unraveling nitrogen fixing potential of endophytic diazotrophs of different saccharum species for sustainable sugarcane growth, International Journal of Molecular Sciences, 23.
https://doi.org/10.3390/ijms23116242

Tian J., Tang M., Xu X., Luo S., Condron L., Lambers H., Cai K., and Wang J., 2020, Soybean (*Glycine max* (L.) Merrill) intercropping with reduced nitrogen input influences rhizosphere phosphorus dynamics and phosphorus acquisition of sugarcane (*Saccharum officinarum*), Biology and Fertility of Soils, 56: 1063-1075.
https://doi.org/10.1007/s00374-020-01484-7

Wen A., Havens K., Bloch S., Shah N., Higgins D., Davis-Richardson A., Sharon J., Rezaei F., Mohiti-Asli M., Johnson A., Abud G., Ané J., Maeda J., Infante V., Gottlieb S., Lorigan J., Williams L., Horton A., McKellar M., Soriano D., Caron Z., Elzinga H., Graham A., Clark R., Mak S., Stupin L., Robinson A., Hubbard N., Broglie R., Tamsir A., and Temme K., 2021, Enabling biological nitrogen fixation for cereal crops in fertilized fields, ACS Synthetic Biology.
https://doi.org/10.1021/acssynbio.1c00049