Journal of Energy Bioscience 2025, Vol.16, No.5, 238-247
http://bioscipublisher.com/index.php/jeb

Research Insight Open Access

Enhancing Biofuel Production by Genetic Engineering of C4 Plant Photosynthesis Pathways

Wenzhong Huang
Biomass Research Center, Hainan Institute of Tropical Agricultural Resouces, Sanya, 572025, Hainan, China
Corresponding email: wenzhong.huang@hitar.org
Journal of Energy Bioscience, 2025, Vol.16, No.5 doi: 10.5376/jeb.2025.16.0023
Received: 04 Aug, 2025
Accepted: 10 Sep., 2025
Published: 24 Sep., 2025

Preferred citation for this article:
Huang W.Z., 2025, Enhancing biofuel production by genetic engineering of C4 plant photosynthesis pathways, Journal of Energy Bioscience, 16(5): 238-247 (doi: 10.5376/jeb.2025.16.0023)

Abstract This study mainly discusses how to use genetic engineering to improve the photosynthesis of C4 plants, thereby increasing the yield of biofuels. C4 crops, such as sugarcane, corn and sorghum, are regarded as good raw materials for biofuels because they can efficiently utilize carbon dioxide and accumulate more biomass. In recent years, genetic engineering methods have developed rapidly. Methods such as CRISPR/Cas editing, synthetic biology, and multi-omics analysis have all been employed to regulate enzymes, transcription factors, and metabolic pathways related to C4 photosynthesis. These methods make photosynthesis more efficient, nitrogen utilization better, and plants more resilient to adverse conditions. However, there are still many problems to be faced in truly applying these achievements to industries. For instance, the adaptive balance of plants in different environments, biosecurity and regulatory requirements, cost input and the difficulty of promotion, etc. In the future, C4 photosynthesis projects may be combined with the transformation of C3 crops. With the addition of systems biology modeling and collaboration among different disciplines, there is an opportunity to cultivate efficient and low-carbon fuel crops. This is also an important direction for promoting sustainable global energy development. The objective of this review is to summarize these advancements and provide references for subsequent research.
Keywords C4 Photosynthesis; Genetic engineering; Biofuel; Synthetic biology; Multi-omics integration

1 Introduction

The global energy crisis is deepening. Fossil fuels not only have a limited quantity but also bring about environmental problems such as greenhouse gas emissions and climate change. These contradictions make it urgent for people to find sustainable alternative energy sources. Biofuels are regarded as an important alternative to fossil fuels due to their high efficiency, economy and relative environmental friendliness. Especially in the context of the continuous increase in population and energy demand, the development of renewable energy is very important for energy security and environmental protection (Cui, 2021; Wang et al., 2021; Zafar et al., 2022).

C4 plants, such as corn, sorghum, sugarcane and miscanthus, play an important role in biofuel production. They have a high photosynthetic efficiency, can make better use of resources, convert solar energy into chemical energy and produce a large amount of biomass. These plants can also grow normally under adverse conditions such as high temperature and drought, and are suitable for cultivation on marginal land, reducing competition with food crops. It is precisely because of its high yield and efficiency that C4 plants are regarded as ideal raw materials for the development of sustainable biofuels (Van Der Weijde et al., 2013; Zafar et al., 2022; Aggarwal et al., 2024).

In recent years, with the unremitting efforts of researchers, molecular biology and genetic engineering have been vigorously developed, providing new opportunities for the improvement of C4 photosynthesis. During the research process, regulating genes related to cell cycle, hormone action, cell wall formation, etc., and using molecular tools such as transcription factors and mirnas on this basis can improve the photosynthetic efficiency of target plants (Von Caemmerer and Furbank, 2016). The use of new technologies such as gene editing and synthetic biology can also more precisely modify the photosynthetic pathway and optimize carbon fixation and energy utilization (Schuler et al., 2016; Ermakova et al., 2020; Nazari et al., 2024; Swift et al., 2024).

The purpose of this research is to sort out the molecular regulatory mechanisms of photosynthesis in C4 plants, introduce the latest progress of genetic engineering in increasing the yield of biofuels, analyze the existing problems at present, and look forward to the future development direction. It is hoped that by integrating multiple factors such as genetics, molecules and the environment, references can be provided for the design and cultivation of efficient bioenergy crops that adapt to future climate change.

2 C4 Photosynthesis and Its Advantages for Biofuel Crops

2.1 Overview of the C4 photosynthetic mechanism

C4 Photosynthesis is a special form of carbon fixation, mainly existing in high-yield crops such as corn, sorghum and sugarcane. Unlike C3 plants, C4 plants complete the CO_2 concentration process in two types of cells, namely mesophyll cells and vascular bundle sheath cells. First, phosphoenolpyruvate carboxylase (PEPC) fixes CO_2 in mesophyll cells into four-carbon compounds, such as oxaloacetic acid. Then these compounds are transported to the vascular bundle sheath cells, where CO_2 is released and handed over to RuBisCO to enter the Calvin cycle. In this way, photorespiration is greatly reduced and the efficiency of carbon fixation is also higher (Ermakova et al., 2020; Cui, 2021; Sahoo et al., 2024; Prasanna et al., 2025) (Figure 1).

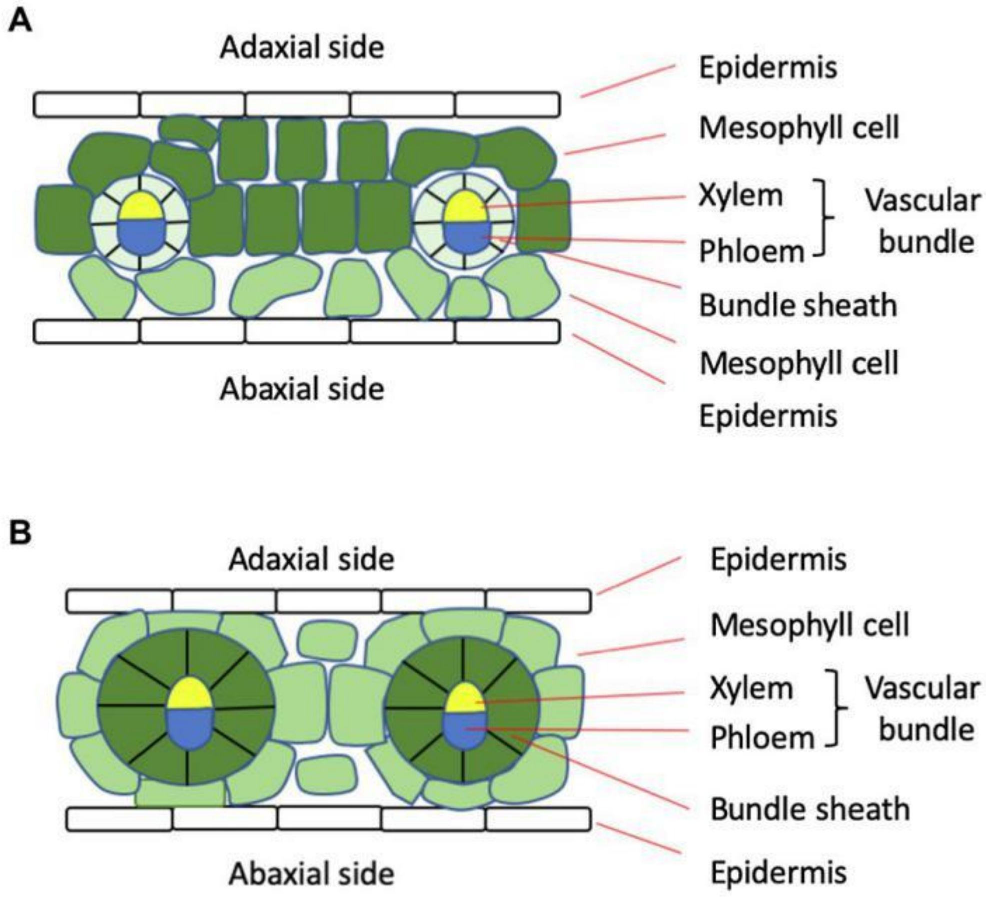

Figure 1 Diagram of the leaf anatomy of typical C3 (A) and C4 (B) plants. In C4 plants, there are two mesophyll cells between neighboring vascular bundles, whereas in C3 plants, there are more than two. Also, the bundle sheath cells in C4 plants are much larger and contain more chloroplasts, as indicated by color intensity (Adopted from Cui, 2021)

2.2 Key advantages for biofuel: higher biomass yield, water use efficiency, nitrogen use efficiency

C4 plants have many advantages in biofuel production. First of all, the output is high. Like corn, sugarcane and sorghum, they have high photosynthetic efficiency and good energy conversion rate, so they can produce more biomass per unit area than C3 plants (Byrt et al., 2011; Mullet, 2017). Theoretically, the photosynthetic energy conversion efficiency of C4 plants can reach 6%, and their actual performance in the field is also better than that of C3 plants (Keller et al., 2021; Wang et al., 2021). The utilization efficiency of water is also very high. Because of the CO2 concentration mechanism, C4 plants can still carry out photosynthesis when the stomata are partially closed, with less water transpiration loss, so they have strong drought resistance (Ghannoum et al., 2010; Ellsworth and Cousins, 2016; Silva-Alvim et al., 2025). The utilization efficiency of nitrogen is also high. C4 plants have a lower demand for RuBisCO than C3 plants, and the nitrogen distribution in their leaves is more reasonable. Therefore, under the same nitrogen input, their CO_2 assimilation rate is higher, which can also reduce fertilizer use (Sharwood et al., 2016; Sahoo et al., 2024; Prasanna et al., 2025). Furthermore, C4 crops can still maintain a good yield in environments such as high temperature, strong light, and drought, and can be planted on marginal land, thereby reducing competition with food crops (Monson et al., 2025).

2.3 Limitations and challenges in natural C4 performance.

Although C4 crops have great potential in the field of biofuels, they themselves also have some limitations. For instance, their photosynthetic efficiency has not yet reached the theoretical maximum value. The actual efficiency in the field is affected by enzyme activity, stomatal regulation and reaction rate under environmental changes (Verhage, 2021). The adaptability to environmental changes is also limited. Under rapid changes in light or extreme temperatures, the photosynthetic regulation of C4 crops will be delayed, resulting in a decrease in carbon assimilation efficiency (Keller et al., 2021; Lee et al., 2021; Wang et al., 2021; Wang, 2024). The difficulty of genetic improvement is also very high. C4 Photosynthesis depends on complex anatomical structures (Kranz structure) and multi-gene regulation. During genetic engineering modification, problems such as cell-specific expression and metabolic coordination need to be addressed (Coelho et al., 2017; Ermakova et al., 2020; Cui, 2021; Sahoo et al., 2024). Another point is that their adaptability to changes in CO_2 concentration is limited. When the concentration of atmospheric CO_2 rises, the resource allocation of some C4 crops does not fully keep up with the changes in the modern environment (Pignon and Long, 2020).

3. Genetic Engineering Strategies to Enhance C4 Photosynthesis

3.1 Improving carbon fixation efficiency

To enhance the carbon fixation efficiency of C4 plants, it is mainly necessary to improve the expression and activity of key enzymes. By enhancing the functions of specific enzymes such as phosphoenolpyruvate carboxylase (PEPC), pyruvate phosphodikinase (PPDK), and NADP-malate enzyme (NADP-ME) through genetic engineering, the concentration and assimilation efficiency of CO_2 can be improved. Meanwhile, Reduce the loss caused by photorespiration (Yadav and Mishra, 2020; Yadav et al., 2020; Nazari et al., 2024). Regulating the expression of carbonic anhydrase (CA) and CO_2 channel proteins helps mesophyll cells better acquire and transport CO2. RuBisCO and its activating enzymes can also be modified to increase their activity in vascular bundle sheath cells and further improve carbon assimilation efficiency (Wang et al., 2021).

3.2 Optimizing light harvesting

The photosynthetic electron transfer of C4 plants takes place respectively in mesophyll cells and vascular bundle sheath cells. By regulating the expression of certain photosystem proteins (such as the cytochrome b6f complex) through genetic engineering, the light energy between the two types of cells can be rationally allocated, thereby enhancing the overall efficiency. Adjusting the size and composition of the optical capture antenna complex, expanding the spectral range of absorption, and reducing energy loss is also an effective measure to improve the utilization rate of light energy (Von Caemmerer and Furbank, 2016; Wang et al., 2021; Nazari et al., 2024).

3.3 Metabolic engineering for biomass accumulation

The final biomass of C4 crops is influenced by multiple metabolic processes such as carbon assimilation, transport and distribution of assimilates, and cell wall synthesis. To enhance the growth rate and yield of C4 crops, researchers typically employ genetic engineering to regulate genes related to the cell cycle, hormone effects (such as auxin and cytokinin), and cell wall synthesis, and then provide assistance with molecular tools like transcription factors and mirnas. In addition to the above methods, enhancing the synthesis and transport of sucrose and allowing more assimilates to be distributed to storage organs can also effectively increase the yield of biofuel raw materials (Cui, 2021; Zafar et al., 2022; Nazari et al., 2024).

3.4 Stress tolerance engineering

To maintain high yields on marginal land, C4 crops need to have stronger stress resistance. By overexpressing antioxidant enzymes, osmotic regulatory substances (such as proline, betaine), heat shock proteins (HSPs), and regulating some transcription factors and mirnas related to stress response, these crops can be made more drought-tolerant, heat-tolerant and salt-tolerant (Nowicka et al., 2018; Yadav and Mishra, 2020; Yadav et al., 2020; Nazari et al., 2024). In addition, C4 photosynthetic genes themselves, such as PEPC and PPDK, can also show the effect of enhancing stress resistance when heterologous expressed.

4 Case Study: Genetic Engineering in C4 Biofuel Crops

4.1 Example: sugarcane genetic engineering for enhanced sucrose and biomass accumulation

4.1.1 Overexpression of PEP carboxylase and rubisco activase

Sugarcane is an important fuel and sugar crop worldwide. In recent years, many researchers have used genetic engineering to modify the key photosynthetic enzymes of sugarcane. Through a series of experiments, researchers ultimately discovered that overexpression of genes such as phosphoenolpyruvate carboxylase (PEPC) and Rubisco activase can enhance CO_2 fixation and carbon assimilation, effectively increasing the sucrose content and biomass in sugarcane. Regulating genes related to sucrose metabolism, carbon allocation and stem maturation using RNA interference and gene editing is also an effective measure to increase sugar production and biomass (Budeguer et al., 2021; Nazari et al., 2024; Brant et al., 2025).

4.1.2 Results: improved photosynthetic rate, higher sucrose content, increased biomass yield

These genetic engineering measures have brought about obvious improvements. The photosynthetic rate of genetically modified sugarcane is higher, and both the sucrose content and biomass yield have increased. For example, sugarcane with overexpression of sucrose phosphosynthase (SPS) not only had significantly increased sucrose content in leaves and stems, but also had better plant height and stem number than the control (Anur et al., 2020; Brant et al., 2025). Furthermore, genetic engineering methods that regulate stem maturity and carbon allocation have further promoted biomass accumulation (Budeguer et al., 2021).

4.2 Example: maize engineering for improved nitrogen use efficiency

4.2.1 Altered expression of GS/GOGAT cycle enzymes

Corn is the model plant of C4 crops, and its nitrogen use efficiency (NUE) directly affects the yield. Nitrogen assimilation and reuse can be enhanced by regulating the expression of key enzymes such as glutamine synthase (GS) and glutamine-2-ketoglutarate aminotransferase (GOGAT) (Lebedev et al., 2021; Liu et al., 2021; Fortunato et al., 2023; Zheng et al., 2025). Meanwhile, studies have found that identifying and overexpressing genes related to NUE (such as ZmNRL1) can also improve the growth performance of maize when nitrogen is insufficient.

4.2.2 Results: enhanced growth under nitrogen-limited conditions

Regulation and modification can enable corn to maintain a high growth rate and yield in a low-nitrogen environment (Lebedev et al., 2021; Fortunato et al., 2023). Overexpression of ZmNRL1 can increase the nitrogen content and chlorophyll level in plants, enhance the tolerance of plants to nitrogen stress, and ultimately achieve higher biomass and yield (Zheng et al., 2025). Optimizing the GS/GOGAT cycle can achieve the goal of improving nitrogen assimilation efficiency and overall productivity (Liu et al., 2021).

4.3 Lessons learned and implications for future biofuel crop engineering

Based on the above two cases, it can be concluded that the genetic engineering modification of key enzymes for photosynthesis and nitrogen metabolism has a positive effect on increasing the biomass, sugar production and resource utilization efficiency of C4 crops (Anur et al., 2020; Lebedev et al., 2021; Fortunato et al., 2023; Nazari et al., 2024; Brant et al., 2025; Zheng et al., 2025). However, Budeguer et al. (2021) pointed out that there are still some technical problems and bottlenecks: the genomes of polyploid crops such as sugarcane are relatively complex, the transgenic efficiency is low, and the stability of traits is insufficient. In the future, multiple strategies such as multi-gene editing, transcription factor regulation, and miRNA targeting need to be combined to jointly enhance photosynthetic efficiency, metabolic distribution, and stress resistance (Ahmad and Ming, 2024) (Figure 2).

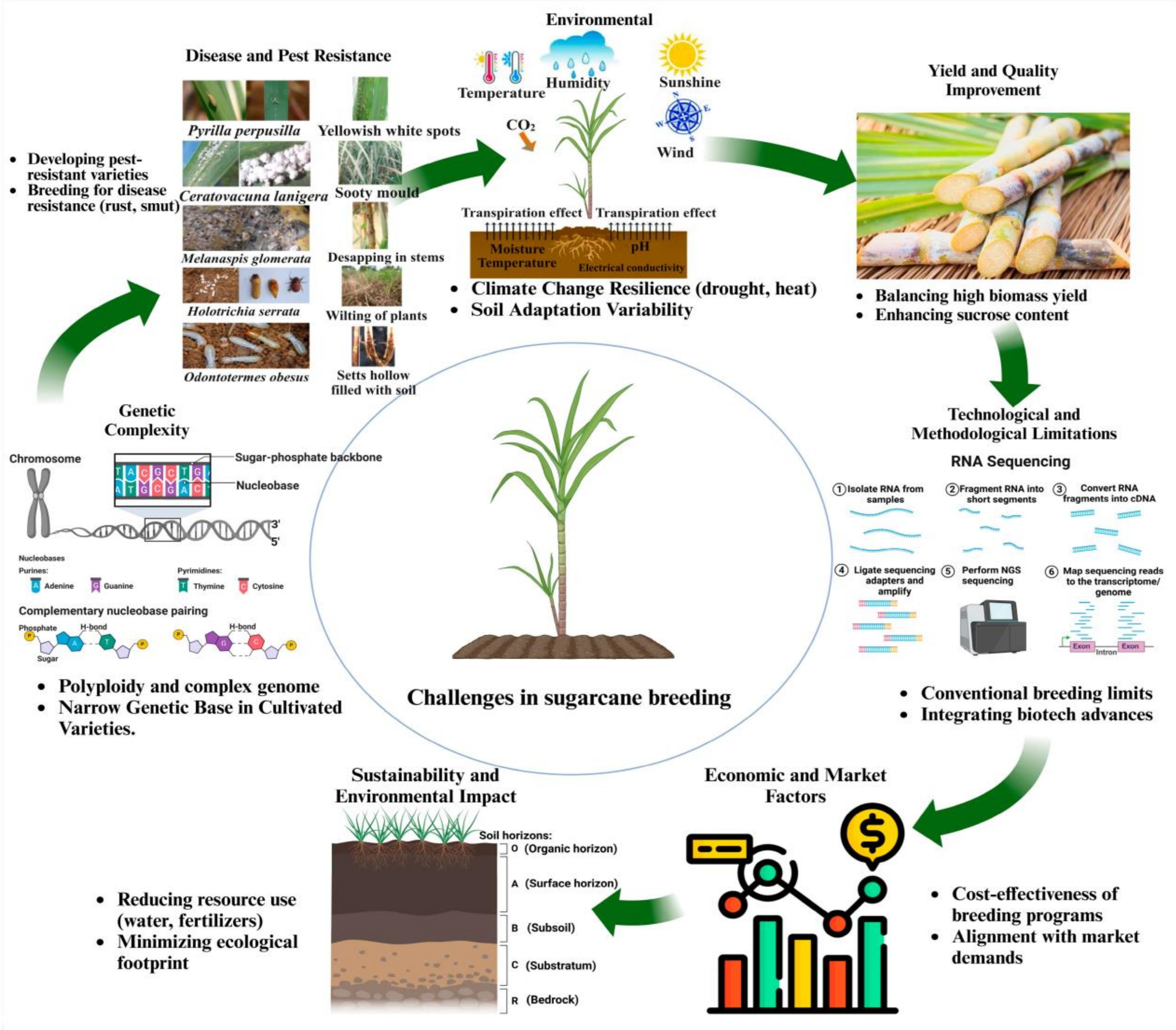

Figure 2 Challenges in sugarcane breeding and their biomass improvement. This figure illustrates the diverse challenges in sugarcane breeding and biomass improvement. Key areas include developing pest-resistant varieties to combat issues like Pyrilla perpusilla and Ceratovacoa lanigera and enhancing disease resistance against rust and smut. Environmental factors such as temperature, humidity, sunshine, and CO_2 levels affect plant health, necessitating climate change resilience and soil adaptation. Yield and quality improvement focus on balancing high biomass yield and enhancing sucrose content. Technological limitations highlight the integration of biotech advances with conventional breeding, particularly through RNA sequencing. Genetic complexity, including polyploidy and a narrow genetic base, poses significant challenges (Adopted from Ahmad and Ming, 2024)

5 Emerging Technologies in C4 Engineering

5.1 CRISPR/Cas genome editing for precise modification of photosynthesis-related genes

The CRISPR/Cas system has become an important tool for modifying the photosynthesis genes of C4 crops. It can achieve gene knockout, knock-in and base replacement, thereby significantly enhancing the efficiency of site-specific modification of key photosynthetic enzymes (such as PEPC, Rubisco, PPDK, etc.) and regulatory factors. CRISPR/Cas9 and its improved forms (such as base editing and guide editing) not only accelerate the trait improvement of C4 crops, but also can simultaneously regulate multiple photosynthetic or metabolic genes through multi-site editing (MGE), thereby promoting the cultivation of multiple traits such as high yield and stress resistance (Abdelrahman et al., 2021). In addition, CRISPR/Cas technology is also making continuous progress in reducing off-target effects, improving editing efficiency and obtaining transgenic offspring (Bao et al., 2019; Chen et al., 2019; Wada et al., 2020).

5.2 Synthetic biology approaches to reconstruct or optimize C4 pathways.

Synthetic biology has brought new ideas for the modification and optimization of the C4 photosynthetic pathway. By modularly assembling multiple enzymes and regulatory elements, the unique carbon concentration mechanism of C4 can be introduced into C3 plants, or the carbon flow distribution can be further improved in C4 crops. Synthetic biology can also design multi-enzyme complexes, introduce artificial regulatory elements, and construct new metabolic pathways to support the improvement of photosynthetic efficiency and biomass accumulation (Hagemann and Hess, 2018; Kumlehn et al., 2018; Abdelrahman et al., 2021).

5.3 Systems biology and computational modeling to predict metabolic flux changes.

Systems biology utilizes genomic, transcriptomic, proteomic and metabolomic data, combined with metabolic network modeling and flux analysis, to predict the impact of genetic engineering intervention on C4 photosynthesis and metabolic processes. Under genome-wide scale modeling, metabolic bottlenecks can be identified, carbon flow allocation can be optimized, and a theoretical basis can be provided for the design of multi-gene editing and synthetic biology (Hagemann and Hess, 2018; Khan et al., 2019).

5.4 Integration of multi-omics (transcriptomics, proteomics, metabolomics) for pathway optimization.

At the current stage, multi-omics analysis (transcriptome, proteome, metabolome) is the main approach to optimizing the C4 photosynthetic pathway. High-throughput data can systematically identify key genes, proteins and metabolites that affect photosynthetic efficiency and biomass accumulation. Combining CRISPR/Cas and synthetic biology tools, these omics data provide solid support for precise regulation and pathway optimization, and can accelerate the molecular design and directed improvement of C4 crops (Hagemann and Hess, 2018; Khan et al., 2019; Alamillo et al., 2023).

6 Challenges and Future Perspectives

6.1 Trade-offs between photosynthesis enhancement and plant growth/fitness.

Enhancing the expression of genes related to C4 photosynthesis can increase carbon assimilation and yield. However, if the photosynthetic pathway is overly strengthened, it may lead to an imbalance in energy and resource distribution, affecting the overall growth of plants. For instance, if key enzymes such as Rubisco are not coordinated with the overall metabolic network, it may lead to a decline in growth tolerance or adaptability (Pradhan et al., 2022; Nazari et al., 2024; Prasanna et al., 2025). Furthermore, both Schuler et al. (2016) and Cui (2021) pointed out that the unique Kranz structure and cell differentiation mechanism of C4 plants are very complex, and physiological bottlenecks or adaptive losses are prone to occur during engineering modification.

6.2 Biosafety, ecological, and regulatory concerns in genetically engineered biofuel crops.

Genetically modified C4 biofuel crops will encounter biosecurity and ecological risks when promoted, such as gene drift, impact on non-target organisms, and disruption of ecosystem balance, etc. The regulatory policies for genetically modified crops vary from country to country, the approval process is complex, and the public acceptance is limited. All these factors have affected the commercialization of genetically engineered C4 crops. Meanwhile, long-term ecological impacts and environmental adaptability still require large-scale field trials and continuous monitoring (Shokravi et al., 2021; Pradhan et al., 2022).

6.3 Scalability and cost-effectiveness of genetically engineered C4 crops for industrial biofuel production.

Although genetically engineered C4 crops have already demonstrated higher biomass and resource utilization efficiency in both laboratory and greenhouse conditions, there are still many challenges to achieving large-scale industrialization of C4 crops, including the stability of multi-gene co-expression, consistency of traits in field environments, compatibility with planting systems, as well as the production and distribution costs of genetically modified seeds. The market price fluctuations of biofuels and the intensity of policy support will affect the economic feasibility of large-scale industrialization of C4 crops (Ambaye et al., 2021; Pradhan et al., 2022).

6.4 Potential synergy with C3-to-C4 engineering in non-C4 biofuel candidates.

Introducing the C4 photosynthetic mechanism into C3 biofuel crops (such as rice and wheat) is an important direction for enhancing global biofuel production capacity in the future. Such projects can not only enhance the efficiency of carbon fixation and nitrogen utilization, but also improve the adaptability of crops in high-temperature and arid environments. However, the C3-C4 project still faces many technical challenges at present, such as differences in anatomical structures, the complexity of gene regulatory networks, and the difficulty of multi-gene collaborative expression. However, with the development of systems biology, synthetic biology and multi-omics techniques, these problems are expected to be broken through (Schuler et al., 2016; Cui, 2021; Pradhan et al., 2022; Prasanna et al., 2025).

7 Conclusion

Over the years, with the unremitting efforts of researchers in related fields, the C4 photosynthesis project has made considerable progress. Through techniques such as molecular breeding, gene editing and synthetic biology, researchers have identified and regulated key genes within plants that affect biomass accumulation, cell cycle, hormone action and cell wall synthesis, significantly increasing the yield and resource utilization efficiency of C4 energy crops such as sugarcane, corn and sorghum. New-generation gene editing tools such as CRISPR/Cas make it possible to precisely modify key enzymes and regulatory factors of photosynthesis. Many studies have shown that introducing C4-related genes into other crops can not only enhance the carbon assimilation of the crops but also improve their stress resistance and adaptability. Now, researchers have attempted to introduce the C4 photosynthetic mechanism into C3 crops and found that this measure can also enhance the photosynthetic efficiency and biomass of the target plants.

The C4 photosynthesis project has laid a molecular foundation for the sustainable development of biofuels. C4 crops have an inherent high efficiency in carbon fixation and resource utilization, which enables them to better cope with adverse situations such as climate change, reducing greenhouse gas emissions and ensuring energy security. In the future, as technologies such as multi-gene editing, synthetic biology and multi-omics integration gradually mature, the photosynthetic efficiency and biomass yield of C4 crops will be further enhanced, thereby promoting the biofuel industry to be more efficient, low-carbon and economical. The new breakthrough from C3 to C4 project can enable more crops to be used for biofuel production and enhance the global bioenergy production capacity.

The continuous progress of C4 photosynthesis engineering is inseparable from the integration of genetics, biotechnology, systems biology and agriculture. Future research needs to pay more attention to aspects such as gene regulatory networks, metabolic flow modeling, as well as field phenotypes and environmental interactions. Only when a complete innovation chain is formed from the laboratory to the field, from molecules to populations, can the efficient molecular design, precise breeding and large-scale application of C4 crops be truly achieved. This interdisciplinary collaboration will also provide solid support for the global production of sustainable biofuels.

Acknowledgments

The author expresses the gratitude to the two anonymous peer researchers for their constructive suggestions on the manuscript.

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

Abdelrahman M., Wei Z., Rohila J., and Zhao K., 2021, Multiplex genome-editing technologies for revolutionizing plant biology and crop improvement, Frontiers in Plant Science, 12: 721203.
https://doi.org/10.3389/fpls.2021.721203

Aggarwal P., Muthamilarasan M., and Choudhary P., 2024, Millet as a promising C4 model crop for sustainable biofuel production, Journal of Biotechnology, 395: 110-121.
https://doi.org/10.1016/j.jbiotec.2024.09.019

Ahmad K., and Ming R., 2024, Harnessing genetic tools for sustainable bioenergy: a review of sugarcane biotechnology in biofuel production, Agriculture, 14(8): 1312.
https://doi.org/10.3390/agriculture14081312

Alamillo J., López C., Rivas F., Torralbo F., Bulut M., and Alseekh S., 2023, Clustered regularly interspaced short palindromic repeats/CRISPR-associated protein and hairy roots: a perfect match for gene functional analysis and crop improvement, Current Opinion in Biotechnology, 79: 102876.
https://doi.org/10.1016/j.copbio.2022.102876

Ambaye T., Vaccari M., Bonilla-Petriciolet A., Prasad S., Van Hullebusch E., and Rtimi S., 2021, Emerging technologies for biofuel production: a critical review on recent progress, challenges and perspectives, Journal of Environmental Management, 290: 112627.
https://doi.org/10.1016/j.jenvman.2021.112627

Anur R., Mufithah N., Sawitri W., Sakakibara H., and Sugiharto B., 2020, Overexpression of sucrose phosphate synthase enhanced sucrose content and biomass production in transgenic sugarcane, Plants, 9(2): 200.
https://doi.org/10.3390/plants9020200

Bao A., Burritt D., Chen H., Zhou X., Cao D., and Tran L., 2019, The CRISPR/Cas9 system and its applications in crop genome editing, Critical Reviews in Biotechnology, 39: 321-336.
https://doi.org/10.1080/07388551.2018.1554621

Brant E., Zuniga-Soto E., and Altpeter F., 2025, RNAi and genome editing of sugarcane: Progress and prospects, The Plant Journal, 121(5): e70048.
https://doi.org/10.1111/tpj.70048

Budeguer F., Enrique R., Perera M., Racedo J., Castagnaro A., Noguera A., and Welin B., 2021, Genetic transformation of sugarcane, current status and future prospects, Frontiers in Plant Science, 12: 768609.
https://doi.org/10.3389/fpls.2021.768609

Byrt C., Grof C., and Furbank R., 2011, C4 plants as biofuel feedstocks: optimising biomass production and feedstock quality from a lignocellulosic perspective, Journal of Integrative Plant Biology, 53(2): 120-135.
https://doi.org/10.1111/j.1744-7909.2010.01023.x

Chen K., Wang Y., Zhang R., Zhang H., and Gao C., 2019, CRISPR/Cas genome editing and precision plant breeding in agriculture, Annual Review of Plant Biology, 70: 667-697.
https://doi.org/10.1146/annurev-arplant-050718-100049

Coelho C., Huang P., and Brutnell T., 2017, Setaria viridis as a model for C4 photosynthesis, Genetics and Genomics of Setaria, 291-300.
https://doi.org/10.1007/978-3-319-45105-3_17

Cui H., 2021, Challenges and approaches to crop improvement through C3-to-C4 engineering, Frontiers in Plant Science, 12: 715391.
https://doi.org/10.3389/fpls.2021.715391

Ellsworth P., and Cousins A., 2016, Carbon isotopes and water use efficiency in C4 plants, Current Opinion in Plant Biology, 31: 155-161.
https://doi.org/10.1016/j.pbi.2016.04.006

Ermakova M., Arrivault S., Giuliani R., Danila F., Alonso-Cantabrana H., Vlad D., Ishihara H., Feil R., Guenther M., Borghi G., Covshoff S., Ludwig M., Cousins A., Langdale J., Kelly S., Lunn J., Stitt M., Von Caemmerer S., and Furbank R., 2020, Installation of C4 photosynthetic pathway enzymes in rice using a single construct, Plant Biotechnology Journal, 19: 575-588.
https://doi.org/10.1111/pbi.13487

Fortunato S., Nigro D., Lasorella C., Marcotuli I., Gadaleta A., and De Pinto M., 2023, The role of glutamine synthetase (GS) and glutamate synthase (GOGAT) in the improvement of nitrogen use efficiency in cereals, Biomolecules, 13(12): 1771.
https://doi.org/10.3390/biom13121771

Ghannoum O., Evans J., and Caemmerer S., 2010, Chapter 8 nitrogen and water use efficiency of C_4 plants, C4 Photosynthesis and Related CO2 Concentrating Mechanisms, 129-146.
https://doi.org/10.1007/978-90-481-9407-0_8

Hagemann M., and Hess W., 2018, Systems and synthetic biology for the biotechnological application of cyanobacteria, Current Opinion in Biotechnology, 49: 94-99.
https://doi.org/10.1016/j.copbio.2017.07.008

Keller B., Zimmermann L., Rascher U., Matsubara S., Steier A., and Muller O., 2021, Toward predicting photosynthetic efficiency and biomass gain in crop genotypes over a field season, Plant Physiology, 188: 301-317.
https://doi.org/10.1093/plphys/kiab483

Khan A., Bilal M., Mehmood S., Sharma A., and Iqbal H., 2019, State-of-the-art genetic modalities to engineer cyanobacteria for sustainable biosynthesis of biofuel and fine-chemicals to meet bio–economy challenges, Life, 9(3): 54.
https://doi.org/10.3390/life9030054

Kumlehn J., Pietralla J., Hensel G., Pacher M., and Puchta H., 2018, The CRISPR/Cas revolution continues: from efficient gene editing for crop breeding to plant synthetic biology, Journal of Integrative Plant Biology, 60(12): 1127-1153.
https://doi.org/10.1111/jipb.12734

Lebedev V., Popova A., and Shestibratov K., 2021, Genetic engineering and genome editing for improving nitrogen use efficiency in plants, Cells, 10(12): 3303.
https://doi.org/10.3390/cells10123303

Lee M., Boyd R., and Ort D., 2021, The photosynthetic response of C3 and C4 bioenergy grass species to fluctuating light, GCB Bioenergy, 14: 37-53.
https://doi.org/10.1111/gcbb.12899

Liu X., Hu B., and Chu C., 2021, Nitrogen assimilation in plants: current status and future prospects, Journal of Genetics and Genomics, 49(5): 394-404.
https://doi.org/10.1016/j.jgg.2021.12.006

Monson R., Li S., Ainsworth E., Fan Y., Hodge J., Knapp A., Leakey A., Lombardozzi D., Reed S., Sage R., Smith M., Smith N., Still C., and Way D., 2025, C4 photosynthesis, trait spectra, and the fast-efficient phenotype, The New Phytologist, 246(3): 879-893.
https://doi.org/10.1111/nph.70057

Mullet J., 2017, High-biomass C4 grasses-Filling the yield gap, Plant Science: An International Journal of Experimental Plant Biology, 261: 10-17.
https://doi.org/10.1016/j.plantsci.2017.05.003

Nazari M., Kordrostami M., Ghasemi-Soloklui A., Eaton-Rye J., Pashkovskiy P., Kuznetsov V., and Allakhverdiev S., 2024, Enhancing photosynthesis and plant productivity through genetic modification, Cells, 13(16): 1319.
https://doi.org/10.3390/cells13161319

Nowicka B., Ciura J., Szymańska R., and Kruk J., 2018, Improving photosynthesis, plant productivity and abiotic stress tolerance - current trends and future perspectives, Journal of Plant Physiology, 231: 415-433.
https://doi.org/10.1016/j.jplph.2018.10.022

Pignon C., and Long S., 2020, Retrospective analysis of biochemical limitations to photosynthesis in 49 species: C4 crops appear still adapted to pre-industrial atmospheric [CO_2], Plant, Cell & Environment, 43(11): 2606-2622.
https://doi.org/10.1111/pce.13863

Pradhan B., Panda D., Bishi S., Chakraborty K., Muthusamy S., and Lenka S., 2022, Progress and prospects of C4 trait engineering in plants, Plant Biology, 24(6): 920-931.
https://doi.org/10.1111/plb.13446

Prasanna C., Sunita K., Satheeshkumar N., Dowarah B., and Singh S., 2025, Engineering C4 photosynthesis pathways into C3 crops to improve nutrient use efficiency: a review, Journal of Experimental Agriculture International, 47(4): 374-381.
https://doi.org/10.9734/jeai/2025/v47i43387

Sahoo J., Mahapatra D., Mahapatra M., Dweh T., Kayastha S., Pradhan P., Tripathy S., Samal K., Mishra A., Dash M., and Nanda S., 2024, Understanding the biochemical, physiological, molecular, and synthetic biology approaches towards the development of C4 rice (*Oryza sativa* L.), Cereal Research Communications, 52: 1459-1471.
https://doi.org/10.1007/s42976-024-00489-4

Schuler M., Mantegazza O., and Weber A., 2016, Engineering C4 photosynthesis into C3 chassis in the synthetic biology age, The Plant Journal: For Cell and Molecular Biology, 87(1): 51-65.
https://doi.org/10.1111/tpj.13155

Sharwood R., Ghannoum O., and Whitney S., 2016, Prospects for improving CO2 fixation in C3-crops through understanding C4-Rubisco biogenesis and catalytic diversity, Current Opinion in Plant Biology, 31: 135-142.
https://doi.org/10.1016/j.pbi.2016.04.002

Shokravi H., Shokravi Z., Heidarrezaei M., Ong H., Koloor S., Petrů M., Lau W., and Ismail A., 2021, Fourth generation biofuel from genetically modified algal biomass: Challenges and future directions, Chemosphere, 285: 131535.
https://doi.org/10.1016/j.chemosphere.2021.131535

Silva-Alvim F., Alvim J., Hibberd J., Harvey A., and Blatt M., 2025, A C4 plant K+ channel accelerates stomata to enhance C3 photosynthesis and water use efficiency, Plant Physiology, 197(2): kiaf039.
https://doi.org/10.1093/plphys/kiaf039

Swift J., Luginbuehl L., Hua L., Schreier T., Donald R., Stanley S., Wang N., Lee T., Nery J., Ecker J., and Hibberd J., 2024, Exaptation of ancestral cell-identity networks enables C4 photosynthesis, Nature, 636: 143-150.
https://doi.org/10.1038/s41586-024-08204-3

Van Der Weijde T., Kamei C., Torres A., Vermerris W., Dolstra O., Visser R., and Trindade L., 2013, The potential of C4 grasses for cellulosic biofuel production, Frontiers in Plant Science, 4: 107.
https://doi.org/10.3389/fpls.2013.00107

Verhage L., 2021, Model behavior: finding out how to increase photosynthesis in C4 crops, The Plant Journal: For Cell and Molecular Biology, 107(2): 341-342.
https://doi.org/10.1111/tpj.15408

Von Caemmerer S., and Furbank R., 2016, Strategies for improving C4 photosynthesis, Current Opinion in Plant Biology, 31: 125-134.
https://doi.org/10.1016/j.pbi.2016.04.003

Wada N., Ueta R., Osakabe Y., and Osakabe K., 2020, Precision genome editing in plants: state-of-the-art in CRISPR/Cas9-based genome engineering, BMC Plant Biology, 20: 234.
https://doi.org/10.1186/s12870-020-02385-5

Wang Y., 2024, Improving photosynthetic efficiency in fluctuating light to enhance yield of C3 and C4 crops, Crop and Environment, 3(4): 184-193.
https://doi.org/10.1016/j.crope.2024.06.003

Wang Y., Chan K., and Long S., 2021, Towards a dynamic photosynthesis model to guide yield improvement in C4 crops, The Plant Journal, 107: 343-359. https://doi.org/10.1111/tpj.15365

Yadav S., and Mishra A., 2020, Ectopic expression of C4 photosynthetic pathway genes improves carbon assimilation and alleviate stress tolerance for future climate change, Physiology and Molecular Biology of Plants, 26: 195-209. https://doi.org/10.1007/s12298-019-00751-8

Yadav S., Rathore M., and Mishra A., 2020, The pyruvate-phosphate dikinase (C4-SmPPDK) gene from suaeda monoica enhances photosynthesis, carbon assimilation, and abiotic stress tolerance in a C3 plant under elevated CO_2 conditions, Frontiers in Plant Science, 11: 345. https://doi.org/10.3389/fpls.2020.00345

Zafar N., Haider F., Fatima M., Ma H., Zhou Y., and Ming R., 2022, Genetic determinants of biomass in C4 crops: molecular and agronomic approaches to increase biomass for biofuels, Frontiers in Plant Science, 13: 839588. https://doi.org/10.3389/fpls.2022.839588

Zheng C., Li H., Liu Y., Huang X., Han J., Luo N., and Li F., 2025, A DUF630 and 632 domains-containing protein, ZmNRL1, acts as a positive regulator of nitrogen stress response in maize, Journal of Experimental Botany, 76(14): 3984-4002. https://doi.org/10.1093/jxb/eraf202