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Genetic Regulation of Photosynthetic Efficiency in Sugarcane: Molecular Basis of C_4 Pathway and Carbon Metabolism

Weichang Wu [1], Zhen Li [2]
1 Biotechnology Research Center, Cuixi Academy of Biotechnology, Zhuji, 311800, Zhejiang, China
2 Hainan Institute of Biotechnology, Haikou, 570206, Hainan, China
Corresponding email: zhen.li@hibio.org
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Abstract Photosynthetic efficiency is a crucial determinant of sugarcane yield and biomass accumulation, particularly in tropical environments where sugarcane, a C_4 plant, thrives due to its high carbon assimilation capacity. This study investigates the genetic regulation of photosynthetic efficiency in sugarcane, focusing on the molecular mechanisms underlying the C_4 pathway and carbon metabolism. We provide a comprehensive overview of C_4 photosynthesis, emphasizing Kranz anatomy and CO2-concentrating mechanisms, and examine the genetic regulation of key enzymes such as PEPC, NADP-ME, and PPDK. The study explores transcriptional and post-transcriptional control, including the role of epigenetic factors and non-coding RNAs, alongside genetic components involved in Rubisco regulation, sugar transport, and carbon partitioning. Furthermore, we analyze the integration of light reactions with carbon metabolism and identify regulatory genes for chlorophyll biosynthesis and photoprotection. Advances in biotechnological tools such as CRISPR/Cas9 and omics-based gene discovery are discussed in this study of improving photosynthetic traits. We also present a case study on elite sugarcane varieties, highlighting the association between gene expression and photosynthetic performance under field conditions. Our findings underscore the need for integrative genetic models and systems biology approaches to optimize photosynthetic efficiency and promote sustainable sugarcane production through targeted genetic innovation.
Keywords Sugarcane; C_4 photosynthesis; Genetic regulation; Carbon metabolism; Photosynthetic efficiency

1 Introduction

Sugarcane is one of the most common cash crops in the fields of many tropical regions, not only because of its high sugar yield, but also because of another factor that is less likely to be overlooked - its photosynthetic efficiency is quite good. People pay attention to it not only because of the demand for sugar, but also because its potential in bioenergy is being magnified year by year. In fact, the photosynthetic efficiency of sugarcane is directly related to how high it can grow, how thick the stem can grow, and how much sugar it accumulates.

In this regard, the difference between different varieties is quite obvious. Some genotypes can always lead in both yield and quality no matter how the environment changes. Therefore, when breeding, we have to consider the issue of “high photosynthetic efficiency”. After all, with the growing demand today, being able to grow more crops with higher yields is likely to solve the dual pressures of food and energy we face (Zafar et al., 2022; Hua et al., 2024; Wei et al., 2024).

When it comes to the photosynthetic mode of sugarcane, it uses the C_4 pathway like corn and sorghum. This mechanism is particularly popular under tropical conditions of high temperature and high light. Compared with common C_3 crops, C_4 has higher photosynthetic efficiency and resource utilization. But don’t expect it to be too idealistic - in reality, when there are problems such as light fluctuations and plants blocking each other, its mechanism will also be affected, such as metabolic interruption, reduced efficiency, etc. Therefore, to truly understand the regulatory logic behind sugarcane photosynthesis, it is not enough to just know that it is C_4, but also to figure out how its internal decarboxylation mechanism is regulated, and even the slightest adjustment of the cell structure may affect the whole body (Sales et al., 2017; Wang et al., 2021; Sales et al., 2023; Wang, 2024).

What this study wants to do is actually to connect the current research clues on the regulation of sugarcane photosynthesis, focusing on the key factors that affect the C_4 pathway and carbon metabolism. For example, what are the genes that control the relevant metabolic enzymes? How are they regulated by upstream networks or transcription factors? What changes will the combination of genetics and environment bring to yield? We are particularly interested in new discoveries in transcriptomics and gene editing in recent years, such as the role of certain miRNAs and TFs in regulating the expression of C_4-related genes. We hope that by summarizing these molecular genetics, physiological mechanisms and breeding strategies, we can provide some new ideas for improving the photosynthetic efficiency and yield of sugarcane and other C_4 crops in the future - after all, the climate is changing, and the sustainable development of agriculture must keep pace.

2 Overview of Photosynthesis in Sugarcane

2.1 Structure and function of C_4 photosynthesis in sugarcane

Not all plants survive by using a single photosynthesis mechanism. Crops like sugarcane use the C_4 pathway, and the structure in its leaves is different, scientifically called the “Kranz structure”. To put it simply, the mesophyll cells (M) and bundle sheath cells (BS) are arranged in a circle like a “nesting doll”. The carbon dioxide in the air is first captured by the PEPC enzyme in the mesophyll cells to produce a four-carbon compound. Then this four-carbon cargo is sent all the way to the bundle sheath cells, where carbon dioxide is released, and Rubisco comes on the scene to pull it into the Calvin cycle (Figure 1). In the final analysis, this "division of labor and cooperation" method helps sugarcane to complete photosynthesis steadily in hot, dry, and light-intensive environments, and the interference of photorespiration is much less (De Oliveira Dal’Molin et al., 2010).

2.2 Comparison of C_3 and C_4 mechanisms in relation to carbon assimilation

C_3 plants and C_4 plants follow two different photosynthesis routes, and the structural differences make them behave very differently in different environments. Take C_3 plants, for example, they arrange the fixation of CO_2 and the subsequent Calvin cycle in the mesophyll cells. The problem is that this arrangement makes it easy for Rubisco to “recognize the wrong object” and accidentally pull in oxygen, resulting in increased photorespiration - especially when the temperature is high and CO_2 is insufficient. C_4 plants like sugarcane cleverly separate these two steps: first use PEPC enzymes to capture CO_2 in the mesophyll cells, and then hand it over to the bundle sheath cells to continue the Calvin cycle. In this way, not only is photorespiration minimized, but the use of water and nitrogen is also more efficient. This also explains why C_4 plants are more “durable” than C_3 plants in hot, drought, and strong light environments (Yadav and Mishra, 2020; Yadav et al., 2020; Cui, 2021).

2.3 Significance of Kranz anatomy and CO_2 concentrating mechanisms

When it comes to C_4 plants, the concept of the “Kranz structure” cannot be avoided. It is not a rare design. High-yield crops such as sugarcane and corn are equipped with this structure. The mesophyll cells and bundle sheath cells are arranged very compactly. In this structure, carbon dioxide is like being centrally supplied and can be efficiently used in the bundle sheath cells, thus preventing Rubisco from being “misled” by oxygen. Interestingly, although most C_4 plants rely on this “cell cooperation” method, there are always exceptions in nature - some species can even complete similar concentration processes with only a single cell. Regardless of the method, the idea behind it is actually the same: how to use carbon dioxide more valuable. With more research on this type of mechanism, perhaps in the future we can “graft” the advantages of C_4 onto C_3 crops. After all, who wouldn’t want to make them more water-saving and efficient?

3 Molecular Regulation of the C_4 Photosynthetic Pathway

3.1 Key genes encoding enzymes of the C_4 cycle

In the final analysis, sugarcane’s C_4 photosynthesis relies on a complex and coordinated enzyme system. Several key enzymes, such as PEPC, NADP-ME and PPDK, play a core role in the whole process. They are not simply “commanded by a unified command”, but are distributed in different cells (mesophyll and bundle sheath), each performing its own duties. For example, the *Pdk* gene is responsible for encoding two PPDK proteins, one in the chloroplast and the other in the cytoplasm, and different regulatory sequences make them “return to their respective positions”. There is also Me1, which produces NADP-ME specifically for C_4 metabolism, and also

relies on regulatory elements at the 5' and 3' ends: one ensures that it is expressed only in bundle sheath cells, and the other ensures that the expression level is high enough. These mechanisms are not simply set, but multi-level and intricately regulate the expression of C_4 enzymes-any slight change may affect the entire carbon fixation process (Taylor et al., 1997).

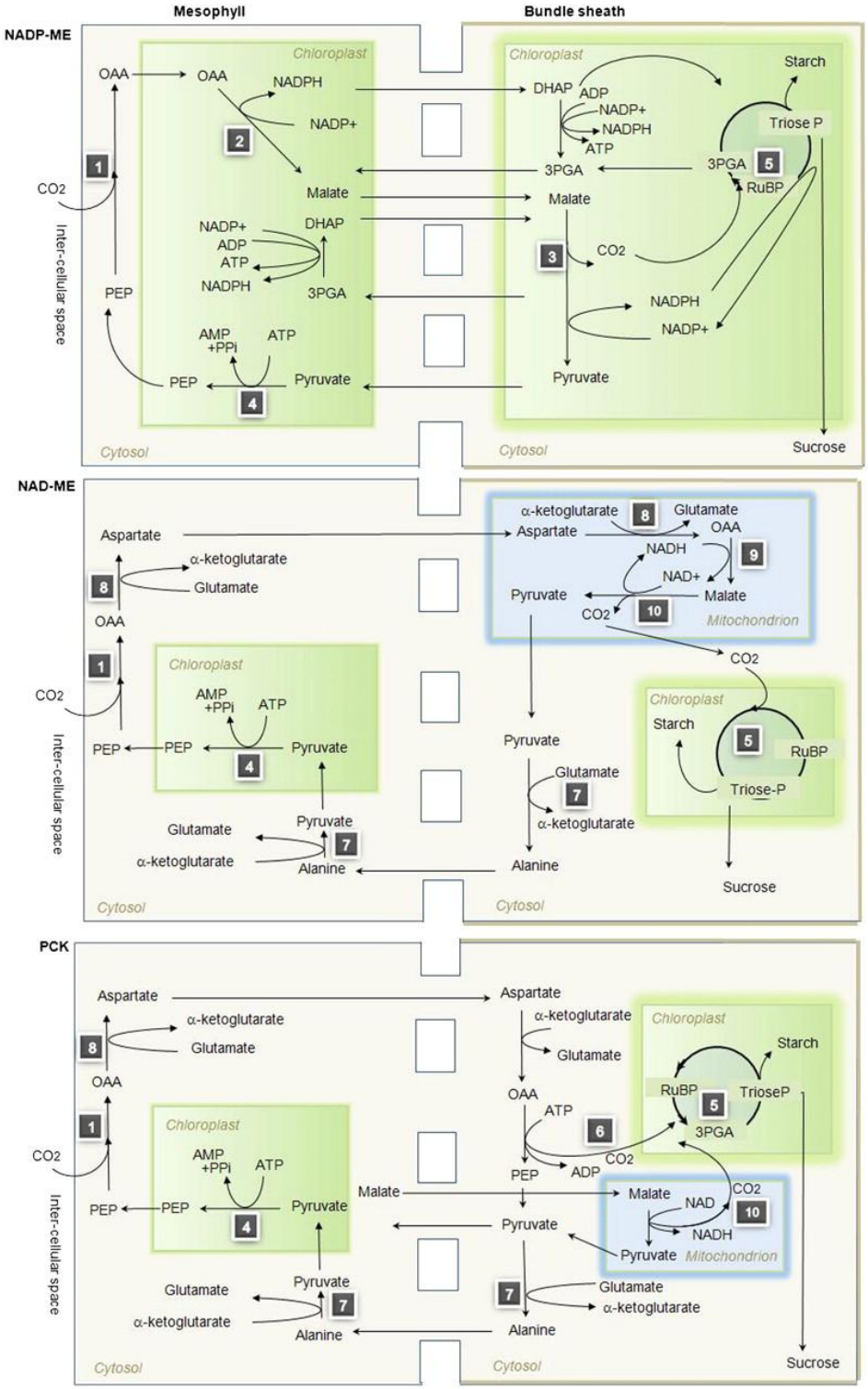

Figure 1 Schematic representation of the photosynthetic metabolism of three C_4 subtypes distinguished according to the decarboxylating enzyme. Numbers refer to enzymes: (1) PEPC, (2) NADP-malate dehydrogenase, (3) NADP-ME, (4) pyruvate-Pi dikinase, (5) Rubisco, (6) PCK, (7) Ala aminotransferase, (8) Asp aminotransferase, (9) NAD-malate dehydrogenase, (10) NAD-ME. Some steps were hidden for the sake of simplicity. 3PGA, 3-Phosphoglycerate; DHAP, dihydroxyacetonephosphate; OAA, oxaloacetate; RuBP, ribulose-1,5-bisphosphate (Adopted from De Oliveira Dal'Molin et al., 2010)

3.2 Transcriptional and post-transcriptional regulation of *C4* genes

Controlling the expression of these genes is not as simple as "turning them on" or "turning them off". Take Pdk for example, its 5' end sequence is like a switchboard, which determines whether it can be efficiently expressed in mesophyll cells. The promoter of its other intron controls the low-level expression in the cytoplasm. The same principle can be seen in Me1-5' controls "where" the expression is, and 3' controls "how much expression". However, this is not all. Some regulatory factors are hidden deeper, such as cis-elements (duons) in exons, which are involved in protein coding on the one hand, and can also affect transcriptional repression in specific cells on the other hand. These "multifunctional elements" are not exclusive to C_4 plants, but are retained in many land plants. It is these hidden regulatory clues that may have played an unexpected role in the evolution of C_4 function (Reyna-Llorens et al., 2018).

3.3 Role of epigenetics and non-coding RNAs in C_4 pathway regulation

When it comes to regulation, we cannot ignore epigenetics. Unlike DNA sequences, which are clear at a glance, epigenetic changes are more like adding some "emotions" to the original "score" - especially the acetylation and methylation of histones, which play a quiet but important role in regulating *C4* genes. Research on corn has found that there are obvious H3K9 acetylation signals in the upstream regions of some photosynthetic genes and C_4-related genes. This phenomenon is particularly concentrated in the so-called R-SUP region (secondary upstream peak), which may be the region that lets genes "know" when to be turned on. Although there are not many studies on sugarcane, from other systems, non-coding RNA, such as "small molecule players" such as miRNA, may also intervene - for example, intervening in chromatin state or controlling the stability of mRNA. In other words, the regulatory network of C_4 may be more complicated than we imagined (Perduns et al., 2015; Morselli and Dieci, 2022).

4 Genetic Control of Carbon Fixation and Transport

4.1 Genes involved in Rubisco activity and Calvin cycle regulation

It is not an exaggeration to say that the enzyme Rubisco is the "opening remarks" of photosynthesis. Without it, CO_2 cannot enter the cycle. But Rubisco does not fight alone. Its activity is regulated by a whole set of transcriptional and post-translational mechanisms. For crops like sugarcane, the expression of Rubisco and a bunch of Calvin cycle-related genes will change when there is shading or excessive sugar accumulation. This shows that its expression is "mutually sensitive" to sugar concentration and reservoir demand, and there may be some kind of kinase pathway involved in the coordination behind it (Figure 2). In addition to Rubisco, enzymes involved in RuBP regeneration cannot be ignored, such as PRK, RPI and RPE-the regulation of these enzymes is actually very critical. They often respond to environmental pressures through changes in redox or metabolic states to ensure that the entire cycle is not "stuck" (Chen et al., 2022; Meloni et al., 2023).

4.2 Sugar transporter genes and carbon partitioning in source-sink dynamics

After carbon fixation, the next question is how to send these sugars to "where they should go". Once the sugar is synthesized in the leaves of sugarcane, it still depends on sugar transporters to be successfully transported to the roots and stems. Especially when photosynthesis is enhanced and the source-sink ratio is unstable, the expression of such transport genes will be significantly upregulated (McCormick et al., 2008). Taking Arabidopsis as an example, sucrose transporters such as SUC and SWEET are responsible for the loading and unloading of sugars in the phloem. The location, timing and even response of these proteins to the external environment are very particular. It can be said that they regulate the efficiency of the "carbon logistics" in the entire plant-how do leaves supply sugar to the roots? How does the storage area adjust the receiving intensity? Behind it, it is inseparable from the work of these transporters at the cellular level (Durand et al., 2017).

4.3 Genetic factors affecting starch and sucrose biosynthesis

Once carbon enters the cell, it is not as simple as converting it on the spot. How the sugar in sugarcane is converted into starch or sucrose depends on the gene expression of the relevant metabolic enzymes. For example, once the source-sink relationship is disrupted, the expression of many metabolic genes will "change accordingly", especially those core enzyme genes that control starch synthesis or sucrose synthesis. Like HXK (hexokinase), its

expression is not only related to the fluctuation of sugar concentration, but also to the efficiency of photosynthesis. This shows that it may be both a "sensor" and a "regulator". In fact, it is not just sugarcane. Studies on other plants have also found that when the gene expression of Calvin cycle enzymes such as PRK and Rubisco changes, not only photosynthesis is affected, but also the synthesis of starch and even lipids will change (Deslandes-Hérold et al., 2022). This kind of discovery makes people realize that if you want to increase the sugar yield of sugarcane, external management alone is not enough, and you have to start from the perspective of regulating key genes.

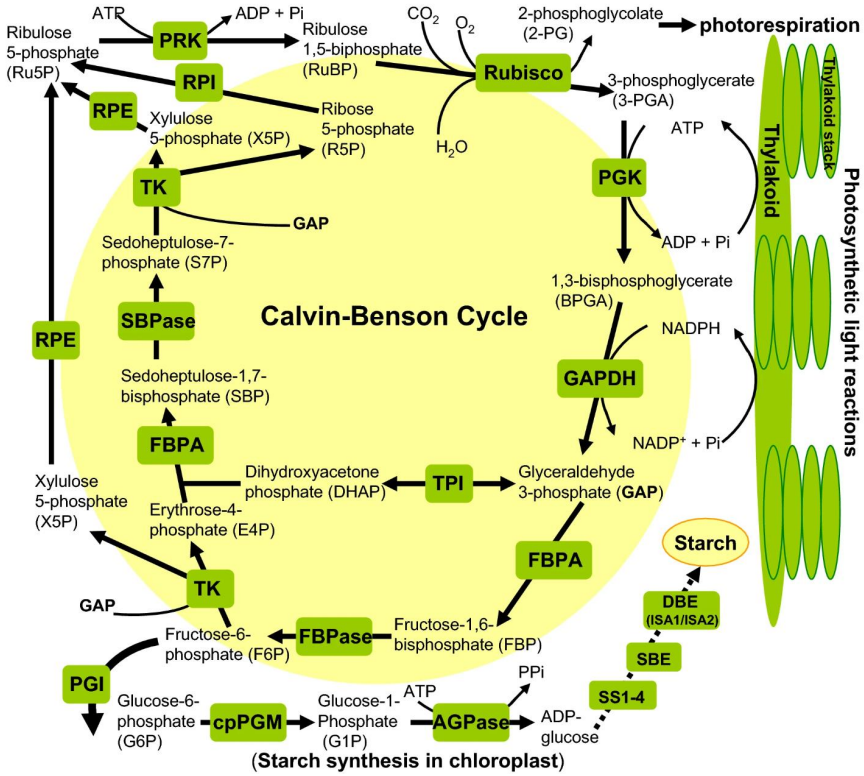

Figure 2 Simplified model of the Calvin-Benson cycle reactions (Adopted from Chen et al., 2022)

5 Integration of Light Reaction and Carbon Metabolism

5.1 Coordination between photosystem function and CO_2 assimilation

In photosynthesis, the light reaction and carbon fixation are not independent of each other. For crops with high light efficiency like sugarcane, the activity of the photosystem and the absorption of carbon dioxide need to be properly coordinated. One cannot run too fast and the other cannot keep up. LHC, or light-harvesting complex, is like installing a "light-enhancing mirror" on the photosystem, which greatly expands the light-harvesting range. With more light energy, there is "power" to fix more carbon dioxide. Especially PSII (photosystem II), whether the structural organization between it and LHC is reasonable directly determines whether the light energy is transmitted smoothly and whether it can be used efficiently. It is not surprising to capture a lot of energy. The key is whether it can be used steadily and turned quickly. This is the fundamental factor affecting the efficiency of carbon fixation (Müh and Zouni, 2020; Lokstein et al., 2021).

5.2 Regulation of photoprotection mechanisms under fluctuating light

The light in nature is not as stable as that in the laboratory. When sugarcane is exposed to the sun in the field, the light intensity changes suddenly. If you are not careful, there will be too much energy to use, which will become a problem. At this time, it is not enough to absorb light desperately, but there must be a way to "release the flood". NPQ (non-photochemical quenching) is like a set of "exothermic valves", which converts excess light energy into heat and discharges it, protecting PSII from oxidation damage. PsbS protein plays the role of "reactor" here, helping plants to quickly adjust their sensitivity to light (Liu et al., 2019). In addition to it, phosphorylation of LHCII protein and structural changes of thylakoid membrane are also part of the system - together they make the

light-harvesting system more flexible: it can gather when it should, and relax when it should, and it can cope with various tosses of strong light, weak light, and variable light (Johnson et al., 2011; Allahverdiyeva et al., 2015).

5.3 Genes controlling chlorophyll biosynthesis and light harvesting complexes

How light energy is captured and how it is distributed after being captured is actually controlled by genes. Genes like *LHCB1* are specifically responsible for producing the main protein in the light-harvesting complex, and the core of the trimer LHCII depends on it. Once this gene has a problem, the chlorophyll content will drop, and even the structure of the thylakoid will be messed up, which will directly affect the light-harvesting process (Vayghan et al., 2021). The light-harvesting system is not a stereotyped template. It has a variety of possible structural changes and can flexibly switch between the two states of "capturing light" or "dissipating energy". Behind these "strategy switches" is the expression regulation of genes related to the light system, which not only affects the photosynthetic efficiency of plants, but also determines whether they can survive under strong light stress (Mascoli et al., 2019; Li et al., 2020).

6 Biotechnological Advances for Improving Photosynthetic Traits

6.1 Use of CRISPR/Cas9 and RNAi to modify key photosynthetic genes

In the field of plant genetic modification, the emergence of CRISPR/Cas9 is indeed a breakthrough. This system can use gRNA to bring Cas9 to the specified DNA location, and then perform "surgery" on that gene, deleting what needs to be deleted and modifying what needs to be modified. Now many studies are using it to modify traits such as nutrition and resistance, and photosynthesis has of course become one of the goals. If those key regulatory genes or enzyme genes can be regulated, photosynthetic efficiency may be pulled up. But then again, the old technology of RNAi has not left the stage, and it is still good at "downregulating" gene expression. Many studies still rely on it to find out what photosynthetic genes are responsible for (Arora and Narula, 2017).

6.2 Transgenic approaches to enhance enzyme activity and carbon assimilation

Not all ideas for improving photosynthetic efficiency rely on "knockout" or "silencing". Sometimes, it is more effective to "stimulate" key enzymes. For enzymes such as PEPC, NADP-ME, and PPDK that participate in the C_4 and Calvin cycles, researchers have overexpressed or optimized their structures through transgenic methods, with the goal of increasing the carbon dioxide fixation capacity. These operations are not new, but as CRISPR/Cas9 becomes more mature, it has become more feasible to modify multiple genes at the same time and selectively activate or inhibit certain genes. Moreover, more precise delivery methods such as ribonucleoprotein complexes (RNPs) have also reduced the probability of "editing errors" (Filippova et al., 2019).

6.3 Omics-driven strategies (genomics, transcriptomics, proteomics, metabolomics) for gene discovery

Without the support of omics technology, many photosynthetic regulatory factors hidden deep in the genome may not be easy to discover. From genes to proteins, and then to the metabolic level, these high-throughput methods can capture everything that happens in plants under different growth conditions. Whose expression level is changing? Which type of metabolite suddenly increased? These data are integrated like a map, pointing out the direction for genetic engineering or breeding. CRISPR is now increasingly used in conjunction with these omics tools, and technologies such as NGS have become routine operations. In this way, which alleles have the potential to improve photosynthesis can also be screened out and verified more quickly (Saini et al., 2023).

7 Environmental and Developmental Factors Influencing Gene Expression

7.1 Effects of temperature, light intensity, and water availability

Plants react to changes in the environment, especially in the expression of photosynthetic genes. Light, as the number one "conductor", has long been proven to regulate many genes through photoreceptors, and can even affect multiple links of transcription and post-transcription (Rasmusson and Escobar, 2007). However, light is not the only variable. Changes in temperature are also quite "capable", directly affecting the level of transcription factors, RNA polymerase activity, and even rewriting the alternative splicing method. When low temperature is combined with light, some genes will be specifically activated - most of these genes are related to antioxidants, pigment synthesis or hormone (such as abscisic acid) production, and are closely linked to plant adaptability.

Water conditions cannot be ignored. Once the water supply is insufficient or extreme temperature differences are encountered, stomatal conductance, Rubisco activity, light system repair, etc. will "fall off the chain", and these adjustments are often completed by transcription factors such as MYB, bZIP, and DREB (Saibo et al., 2009).

7.2 Stage-specific expression of photosynthesis-related genes

Gene expression is not a "static" thing, especially those genes related to photosynthesis, which show strong dynamics in different growth stages and environmental conditions. Take the seedling stage as an example. When plants just "open their eyes" from the darkness, light signals and temperature signals have begun to cooperate, and through regulatory mechanisms such as the HY5-PIF module, they step by step promote the activation of genes for chlorophyll and carotenoid synthesis. This is the key stage for them to switch from dependence to autotrophy (Toledo-Ortiz et al., 2014). However, in addition to rapid responses, there are also some "slow variables" at work. The acquisition of traits such as stress tolerance often depends on the long-term maintenance of transcriptional states, which is closely related to the gene expression memory mechanism we are familiar with (Jarad et al., 2020). What's more complicated is that the nuclear genome and the chloroplast genome must maintain communication, and they use anterograde and retrograde signals to ensure that the expression rhythm matches the developmental and environmental needs (Berry et al., 2013).

7.3 Hormonal signaling pathways regulating photosynthetic capacity

When plants cope with environmental stress, they rely not only on regulation at the gene level, but also on hormone signals as another "central control system". Hormones such as abscisic acid (ABA) and auxin not only regulate gene expression, but also act as "indirect promoters" of photosynthetic capacity. Especially in the context of strong light or low temperature, the synthesis and signal transduction of ABA will follow the environment and activate a group of genes related to protection and adaptability in a timely manner (Soitamo et al., 2008). Transcription factors such as PIF4 and SEUSS (SEU) are like "translators" who are responsible for integrating light, temperature and hormone signals together and then transmitting them to downstream synthesis or response genes, thereby affecting plant growth and photosynthesis (Huai et al., 2018; Huang et al., 2019). These hormone pathways do not operate alone, they will "negotiate" with external environmental conditions to ultimately fine-tune the photosynthetic efficiency and adversity adaptability of plants.

8 Case Study: Gene Regulation and Yield Performance in Elite Sugarcane Varieties

8.1 Identification of high-expression C_4 gene clusters in commercial hybrids

Not all genes that have been preserved have become the protagonists, but in the evolution of C_4 plants, a group of genes that were originally expressed at high levels in ancestral non-C_4 plants later became the "main force" of the C_4 pathway in modern sugarcane hybrids. In this type of gene cluster, in addition to familiar faces such as PEPC, PPDK, and NADP-ME, other members involved in light response, sugar metabolism, transcriptional regulation, and metabolite transport can also be seen (Moreno-Villena et al., 2017). Their high expression in leaves is not accidental, and it is likely that they are "going with the flow" in the process of forming and strengthening the C_4 cycle. In breeding practice, the expression levels of these genes have gradually been "pushed up", and many excellent varieties have come to the forefront thanks to them.

8.2 Correlation between gene expression profiles and photosynthetic rates under field conditions

Performance in the greenhouse is certainly important, but the field is the "real battlefield". Many studies have found that under actual planting conditions, there is a clear relationship between those C_4-related metabolic enzyme genes - once the expression level is increased - and the improvement of photosynthetic capacity. For example, in hybrids of corn and sorghum, the expression of key enzymes such as carbon assimilation enzymes can even exceed that of the parents. This "non-additive" expression is often accompanied by a simultaneous increase in net photosynthetic rate, stomatal conductance, and transpiration rate, and it can indeed be reflected in grain weight and total biomass (Li et al., 2020). These data are actually illustrating a problem: when looking at photosynthetic efficiency and yield, you might as well start with the expression spectrum, which may be a very reliable "early warning system" (Zhao et al., 2024).

8.3 Genetic markers associated with enhanced carbon assimilation and sugar yield

Not all varieties with excellent performance can explain "why they are good". However, we can now identify some key genetic markers that are directly related to highly expressed C_4 pathway genes and carbon assimilation enzymes. Sites related to enhanced activity of PEPC, PPDK, and NADP-ME, as well as regulatory elements involved in sucrose and starch metabolism, have been repeatedly verified in some excellent hybrid sugarcanes (Figure 3) (Ding et al., 2015). What's more interesting is that behind these high expressions, there are often clustered gene regulatory regions supporting them, which provides a very practical "entry point" for marker-assisted selection and precision breeding. If we can make further progress in this regard, there may be a lot of room for improvement in sugarcane yield and carbon utilization efficiency.

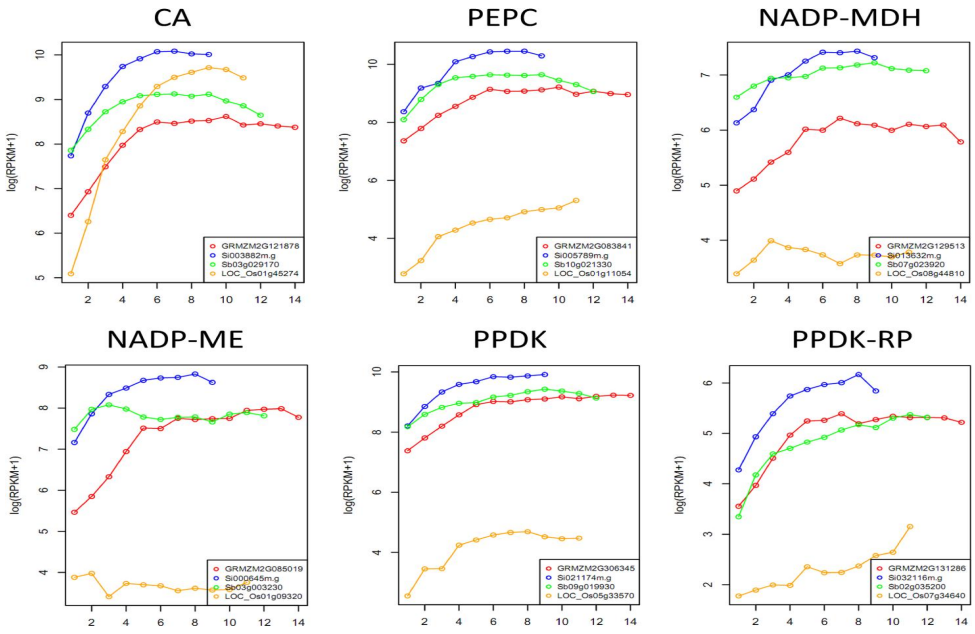

Figure 3 Expression pattern of classical *C4* genes in four species (Adopted from Ding et al., 2015)

Image caption: Gene IDs were plotted with different colors, e.g., red, blue, green and yellow for maize, green foxtail, sorghum and rice, respectively. CA: carbonic anhydrase, PEPC: phosphoenolpyruvate carboxylase, NADP-MDH: NADP-malate dehydrogenase, NADP-ME: NADP-malic enzyme, PPDK: pyruvate orthophosphate dikinase, and PPDK-RP: PPDK regulatory protein (Adopted from Ding et al., 2015)

9 Future Perspectives and Research Directions

9.1 Need for integrative genetic models linking gene regulation to whole-plant productivity

Sometimes it is difficult to explain why sugarcane grows fast or produces high sugar by simply looking at whether a gene is expressed well. What is really valuable for reference is the integrated model that can put gene regulation into the performance of the whole plant. Systems biology has actually been doing this for a long time - it is not a single breakthrough, but relies on multi-omics data and computational simulation to pull factors that affect photosynthesis, stress resistance, nutrient efficiency, etc. into a "network diagram" (Kumar et al., 2015). These models have an advantage, that is, they can "artificially" intervene in the plant system, and then observe the feedback of genes or proteins, so as to predict whether a certain gene mutation will make the plant grow faster and produce more. Compared with those traditional methods, integrated models can explain more carefully, and factors such as population competition, species diversity, and environmental gradients that are difficult to quantify in the field can also be included in the analysis range (Grace et al., 2016). If you want to breed high-yield and "smart" sugarcane, this prediction framework from genes to the whole plant is basically unavoidable.

9.2 Potential of synthetic biology in designing high-efficiency C_4 pathways

Synthetic biology has been a bit "hot" in recent years, but not everyone really understands its potential. It is not simply to move the *C4* genes of other plants, but to redesign a whole set of operating logic from regulatory networks, metabolic pathways to gene expression levels. Operations such as constructing synthetic gene circuits,

customizing promoters or regulatory elements are aimed at making gene expression more controllable and efficient (Bashor and Collins, 2018; Huang et al., 2021). Now, methods such as combinatorial DNA assembly and transplastomics have also been introduced, which can make prototype plant construction faster (Jackson et al., 2021). However, if you want to successfully transfer the C_4 mechanism into C_3 crops, it is not as simple as changing the engine, because C_4 needs to coordinate anatomical structure and cell-specific expression, which must be systematically understood (Schuler et al., 2016). In the final analysis, synthetic biology is like opening a toolbox for "reprogramming plants". Although the technology is complex, the idea is actually one: let the plant operate efficiently in the way you set it (Kassaw et al., 2018).

9.3 Importance of multi-environment field validation and systems biology

No matter how well it is described in the book or how beautiful the laboratory data is, once it is exposed to heat, rain, or wind in the field, many effects will become unstable. Therefore, whether it is traditional genetic engineering or synthetic biology design, the step of "multi-environment verification" cannot be avoided in the end. Especially under different combinations of water, temperature, and soil nutrients, how genes and the environment interact and how resources are allocated, once these factors are superimposed, plant performance becomes difficult to predict (Liao et al., 2017; Sickle et al., 2020). To understand these, field yield measurement alone is not enough, and systems biology must keep up - after the integration of omics data, the model can be closer to the real growth dynamics (Cui, 2021). And those "good traits" that have been verified can remain stable in different locations and years. This is the premise for the real implementation of smart breeding.

10 Conclusion

The high photosynthetic efficiency of sugarcane is largely due to its C_4 photosynthetic pathway. But the operation of this pathway is far from being as simple as it seems. It involves a whole set of intricate genetic regulatory networks. Recent transcriptome and small RNA studies have actually revealed a little "unpopular" discovery - in many cases, miRNA does not directly control *C_4* genes, but "indirectly intervenes" through some transcription factors such as the GRAS family. These regulatory relationships further affect chlorophyll synthesis, carbon fixation, and a series of metabolic processes, which will eventually be reflected in the strength of photosynthesis. After analyzing the transcriptome and metabolome data together, researchers have unearthed thousands of genes and metabolites related to carbon fixation, sugar metabolism, and stress resistance. These achievements are not a simple accumulation of data, but a "gene map of excellent sugarcane varieties" for us. In other words, if you want to breed good varieties, you have a direction to start with.

In terms of breeding methods, methods such as MAS, GS, and genetic engineering are no longer unfamiliar. Genomic selection is particularly worth mentioning. It can significantly speed up the breeding process and improve the accuracy of high-quality clone screening, especially with the support of high-throughput phenotyping methods. Of course, the polyploid genome of sugarcane does make things a bit tricky, but now gene editing technology is becoming more and more mature, and CRISPR is no longer just a "showmanship" in the laboratory, but can really move to the "core position" of regulating photosynthesis, sugar accumulation and stress resistance genes.

It can be said that by combining omics data with modern breeding tools, we have taken a key step towards new high-yield and stress-resistant sugarcane varieties. In the future, if we want to grow sugarcane stably and produce high yields, we cannot do without the support of these genetic and biotechnologies. Especially in the face of climate uncertainty, resource constraints and other challenges, precision editing, systematic omics analysis and intelligent breeding strategies are becoming the way to deal with it. Of course, no matter how good the laboratory data is, it has to return to the field for testing. Only by continuing to study the photosynthetic regulatory network in depth, combined with large-scale phenotyping technology and multi-environment testing, can we truly transform these "potential genes" into real yields and resistance. In the long run, these innovations will not only help us meet the world's growing demand for sugar and bioenergy, but also drive agricultural production towards a more sustainable direction.

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Conflict of Interest Disclosure

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

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