

Research Insight

Open Access

Research Insights into Photosynthetic Gene Regulation in Rice for Yield Improvement

Mingliang Jin, Danyan Ding ✉

Institute of Life Sciences, Jiyang College of Zhejiang A&F University, Zhuji, 311800, Zhejiang, China

✉ Corresponding email: 723822780@qq.comRice Genomics and Genetics, 2025, Vol.16, No.1 doi: [10.5376/rgg.2025.16.0004](https://doi.org/10.5376/rgg.2025.16.0004)

Received: 20 Dec., 2024

Accepted: 30 Jan., 2025

Published: 16 Feb., 2025

Copyright © 2025 Jin and Ding, This is an open access article published under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Preferred citation for this article:Jin M.L., and Ding D.Y., 2025, Research insights into photosynthetic gene regulation in rice for yield improvement, Rice Genomics and Genetics, 16(1): 36-49 (doi: [10.5376/rgg.2025.16.0004](https://doi.org/10.5376/rgg.2025.16.0004))

Abstract This study explored the regulatory mechanisms of rice photosynthesis genes and their potential for improving yield, and comprehensively analyzed the genetic and molecular regulation of photosynthesis, including processes such as light capture, carbon fixation, and energy conversion. The results showed that through transcriptomics, proteomics, and CRISPR gene editing, key regulatory genes and pathways that directly affect photosynthesis efficiency, such as Rubisco activating factors and chlorophyll biosynthesis genes, were identified. Environmental interactions and epigenetic factors also have important effects on these processes, providing possibilities for adaptive breeding strategies. The use of photosynthesis gene regulation offers great potential for sustainable rice cultivation. Future research should focus on multi-omics integration and environmental adaptability. This study aims to provide a scientific basis for the realization of a comprehensive yield improvement strategy.

Keywords Photosynthetic gene regulation; Rice yield improvement; Photosynthetic efficiency; Genome editing; Molecular breeding

1 Introduction

More than half of the world's population eats rice every day (Huang, 2024), but it is not easy to increase yields. There are many challenges in growing rice now. We need to produce more food while considering environmental issues. For example, excessive use of nitrogen fertilizer not only pollutes the environment, but is also bad for human health (Wei et al., 2022). Climate change has made the situation more complicated. Drought, high temperature and other extreme weather conditions are becoming more and more frequent, which directly affects the photosynthesis of rice, and the yield will naturally not increase (Ambavaram et al., 2014). Moreover, the yield is related to many genes, which is particularly difficult to study (Adachi et al., 2020). It is quite contradictory. Now we need to increase production, reduce the use of fertilizers, and deal with increasingly unreliable weather. Breeding experts are also troubled. After all, these problems cannot be solved by changing varieties.

When it comes to increasing rice yields, photosynthesis is definitely the key. It's like a factory production line. If the efficiency is high, the yield will naturally increase. Some recent studies are quite interesting. For example, regulating the OsDREB1C gene can not only enhance photosynthesis, but also improve nitrogen fertilizer utilization. At the best, it can increase yield by nearly 70% (Wei et al., 2022). However, there are also studies that go the other way. For example, knocking out the NRP1 gene can actually improve photosynthesis efficiency and make the plants grow stronger (Chen et al., 2020). Even more amazing is directly transferring corn genes into rice, or adjusting some microRNAs, which also has good results (Pan et al., 2018; Li et al., 2020). Of course, these methods have their own ways. Some are suitable for this variety, and some are suitable for that environment. But in general, starting with photosynthesis is indeed a good way. Although the operation is quite complicated, these investments are still worthwhile in order to produce more food.

Let's talk about the regulation of rice photosynthesis genes. In recent years, researchers have made great efforts in this area and discovered many key genes that can increase yield (Wei et al., 2022). From transcription factors to microRNAs, there are quite a few targets that can regulate photosynthesis. It's interesting to say that some genes can increase yields by overexpression, such as OsDREB1C; some genes need to be knocked out to have an effect,

such as NRP1 (Chen et al., 2020). But the most surprising thing may be that transferring corn genes to rice actually works (Pan et al., 2018). These findings provide new ideas for breeding. Although the specific mechanism remains to be studied, improving photosynthetic efficiency through gene regulation is indeed a good way to achieve high yield and environmental protection. Of course, in actual operation, factors such as variety adaptability and environmental impact must also be considered. But in general, this path is worth continuing to explore.

2 Photosynthetic Pathways in Rice

2.1 C₃ photosynthesis mechanism in rice and its limitations

To be honest, the C₃ photosynthetic pathway used by crops like rice is not very efficient. It mainly relies on the enzyme Rubisco to fix carbon dioxide and produce 3-phosphoglyceric acid (Sen et al., 2016). But the problem is that Rubisco sometimes recognizes the wrong object - fixing oxygen as carbon dioxide, which is the phenomenon of photorespiration. Especially in the hot sun or high temperature environment, this "misidentification" situation is more serious (Paul, 2021). Many major rice-producing areas happen to have such climatic conditions, resulting in a large amount of carbon and energy being wasted (Zhang et al., 2022). It's a pity to say that it obviously absorbs a lot of light energy, but not much can be used for growth in the end. In fact, there is nothing wrong with the C₃ pathway itself, but it is a bit unsuitable for rice. After all, rice is mainly grown in tropical and subtropical regions, and often encounters high temperature and strong light weather. This also explains why some temperate crops do not suffer so much when using the C₃ pathway.

2.2 Key enzymes involved in photosynthesis

When it comes to rice photosynthesis, there are several enzymes that are particularly critical. Rubisco is a familiar enzyme to everyone. It is a "model worker" in chloroplasts and is responsible for converting carbon dioxide into organic matter (Zhang et al., 2019; Perveen et al., 2020). Although its efficiency is sometimes unstable, it is indeed the main force of photosynthesis. Interestingly, PEP carboxylase, an enzyme that usually works in C₄ plants, also works well after overexpression in rice (Figure 1). Studies have shown that it can increase the photosynthetic rate of rice, and the yield also increases (Sen et al., 2016). Carbonic anhydrase is also very important. It helps convert carbon dioxide into bicarbonate, which is considered to be a forerunner for photosynthesis (Zhou et al., 2023). In fact, these enzymes have their own division of labor. Rubisco focuses on carbon fixation, while PEP carboxylase and carbonic anhydrase assist from different angles. Although their working mechanisms are different, their goals are the same-to enable rice to photosynthesize more efficiently. Of course, the effects may vary depending on the species and environment.

2.3 Comparative overview of C₃ and C₄ pathways and implications for rice

C₃ plants such as rice and C₄ plants such as corn have very different ways of photosynthesis. Rice uses Rubisco to fix carbon dioxide directly in mesophyll cells, which is simple and direct (Sen et al., 2016). However, when it is sunny or hot, this system is prone to problems - Rubisco starts to "recognize the wrong person" and fixes oxygen as carbon dioxide, wasting energy (Paul, 2021). Corn is much smarter. It has a "carbon dioxide express system". First, PEP carboxylase is used in mesophyll cells to package carbon dioxide into four carbon compounds, and then it is transported to the vascular bundle sheath cells for release (Ermakova et al., 2020). In this way, the carbon dioxide concentration in the working environment of Rubisco is high, and it is naturally less likely to make mistakes. So you see, in hot and strong light environments, C₄ plants such as corn are obviously more advantageous (Zhang et al., 2022). Their mechanism is equivalent to an "insurance" for photosynthesis. Although it is a bit complicated, it does work. But then again, it is not realistic to really switch to the C₄ pathway for rice, as evolution does not happen overnight.

Recently, there is an interesting direction in rice research-"borrowing" the advantages of corn's C₄ photosynthesis. Scientists have tried many methods, such as making rice produce more enzymes unique to C₄ plants (Sen et al., 2016). Experiments have shown that enzymes such as PEP carboxylase and carbonic anhydrase can indeed improve photosynthetic efficiency, and the yield has also increased. However, enzymes alone are not enough, and the internal structure of the leaves must also be modified. Increasing the density of leaf veins and optimizing the vascular bundle sheath cells, these anatomical adjustments also seem to have potential (Ermakova et al., 2020). Although it is not realistic to completely turn rice into a C₄ plant, it is still feasible to borrow some of its

characteristics. In fact, this idea is like renovating an old house - retaining the main structure and only upgrading the most critical parts. Although the project is quite complicated, judging from the experimental results, it is indeed worth continuing to explore (Ermakova et al., 2019). After all, in the context of climate change, improving the photosynthetic efficiency of rice is becoming more and more important.

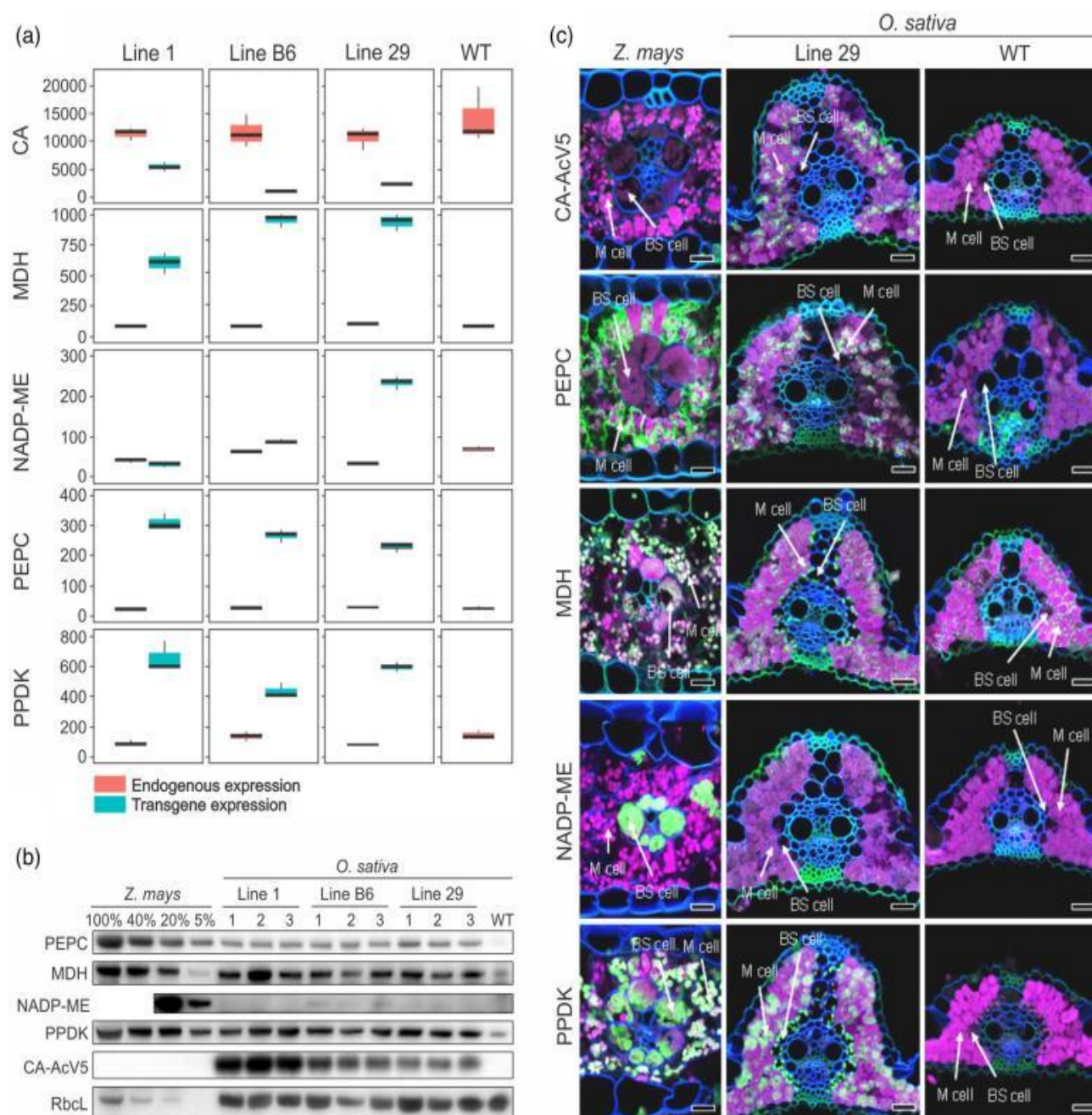


Figure 1 Expression of C₄ enzymes in *O. sativa* (Adopted from Ermakova et al., 2020)

Image caption: (a) Transcript abundance (in transcripts per million) of *Z. mays* transgenes and orthologous to them endogenous genes in wild-type (WT) and three transgenic rice lines. CA, carbonic anhydrase; MDH, NADP-malate dehydrogenase; NADP-ME, NADP-dependent malic enzyme; PEPC, PEP carboxylase; PPK, pyruvate orthophosphate dikinase. Mean±SD, *n*=3 biological replicates. (b) Immunodetection of proteins in leaf extracts loaded on leaf area basis. *Z. mays* leaf extract dilution series was used for relative quantification; three plants from each transgenic line were analysed. Signal from RbcL (the large subunit of ribulose biphosphate carboxylase oxygenase) was used as loading control. (c) Confocal micrographs of protein localization on leaf cross-sections. Fluorescence signals are pseudo-coloured: green-protein of interest labelled with secondary antibodies conjugated with Alexa Fluor 488; magenta-chlorophyll autofluorescence; blue-calcofluor white-stained cell walls. BS, bundle sheath; M, mesophyll. Scale bars=20 μm (Adopted from Ermakova et al., 2020)

3 Genetic Regulation of Photosynthesis

3.1 Overview of photosynthetic genes and regulatory networks

Photosynthesis is critical for rice, but the mechanism that regulates this process is not that simple. It is not that a single gene can do the job, but it involves a complex network of genes and transcription factors. Together, these components affect light capture, carbon fixation, and how energy is converted and utilized. Chlorophyll binding proteins, subunits of the photosystem, and enzymes in the Calvin-Benson-Bassham cycle do not work independently, but there is a coordinated regulatory process behind them. For example, studies have found that if the mEmBP-1 transcription factor from corn is overexpressed in rice, many key genes can be "fully powered", including those that control chlorophyll a and b binding proteins, subunits of the photosystem I and II, and Calvin cycle enzymes. The expression levels are all increased, resulting in enhanced photosynthesis and increased yields (Perveen et al., 2020).

But this is not the only case. Others have found that when the GLK transcription factor in corn is expressed in rice, it not only improves chloroplast development, but also improves photosynthetic efficiency, ultimately leading to a double increase in biomass and yield (Li et al., 2020; Yeh et al., 2021). So, although these gene regulatory networks are complex, once the rules are understood, they can bring very real benefits.

3.2 Role of transcription factors in regulating photosynthetic pathways

In the matter of photosynthesis, transcription factors play a key role, but they do not act alone. They regulate gene expression by binding to specific DNA fragments, and there is actually a very sophisticated mechanism behind this. For example, there is a transcription factor called OsDREB1C, which is induced and activated under strong light or low nitrogen conditions. It affects not only photosynthesis, but also nitrogen utilization and even flowering time. Studies have found that if this gene is expressed more in rice, the photosynthetic efficiency, nitrogen utilization rate and yield of the plant will increase (Wei et al., 2022).

Of course, this is not the only factor with similar effects. Transcription factors like HYR (high-yield rice) are also quite special. They can directly promote the enhanced expression of photosynthesis-related genes, and maintain good carbon metabolism and yield even in poor environmental conditions (Ambavaram et al., 2014). Another example is that although the GLK transcription factor comes from corn, if it is introduced into rice, it can also increase the chlorophyll content and light capture efficiency, and the final result is also stronger photosynthesis and higher yield (Li et al., 2020; Yeh et al., 2021). Therefore, sometimes "borrowing power" from other species can also bring unexpected results.

3.3 Gene expression and regulation under varying environmental conditions

Rice is very sensitive to the environment, especially in photosynthesis. Stress such as light intensity, nitrogen content, or drought and high temperature will affect the expression of photosynthetic genes. Interestingly, when there is sufficient light or low nitrogen, the expression of the transcription factor OsDREB1C will increase significantly, which will improve the photosynthetic efficiency and nitrogen utilization ability of rice (Wei et al., 2022). But not all cases can rice "cope calmly". Studies have shown that most of the time, photosynthesis-related genes will be downregulated regardless of abiotic or biotic stress, which reminds us that improving stress resistance is an unavoidable direction (Cohen and Leach, 2019). However, there are ways, such as the HYR transcription factor, which can activate a whole set of photosynthesis-related genes under high temperature and drought conditions, thereby stabilizing rice yields (Ambavaram et al., 2014).

In addition, some studies are also trying to use regulatory factors such as GLK or miR408 to find ways to improve photosynthesis efficiency under various environments, and the goal is of course to increase yield (Figure 2) (Pan et al., 2018; Yeh et al., 2021). So, environmental pressure does make rice difficult, but it is still possible to "turn the tables" by regulating gene expression.

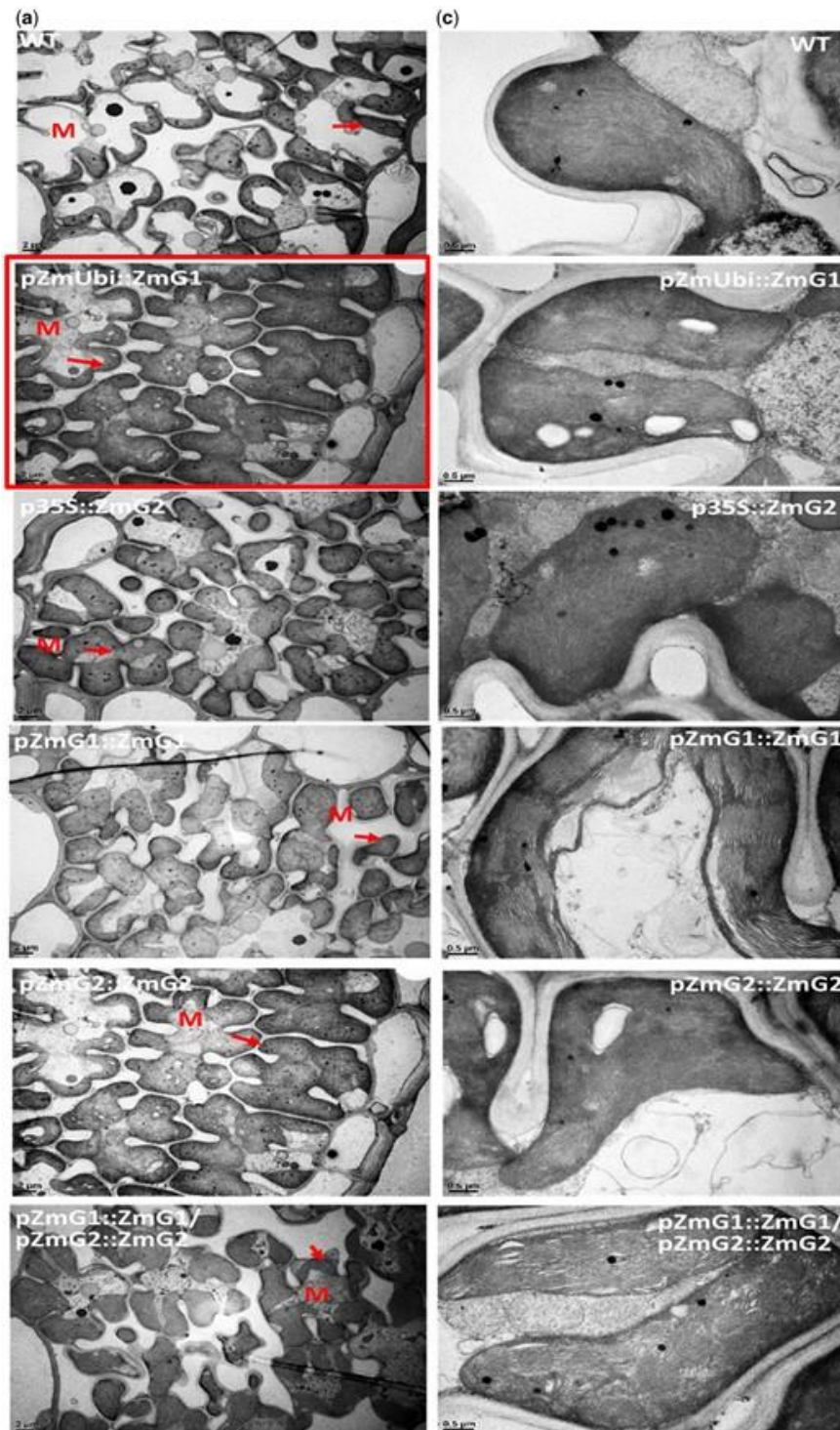


Figure 2 Transmission electron micrographs of flag leaf cross-sections of WT and five GLK transgenic rice plants (Adopted from Yeh et al., 2021)

Image caption: A, B, Chloroplasts in M (A) and BS (B) cells of the WT and five GLK transgenic rice plants, with arrows pointing to representative chloroplasts. C and D, Enlarged micrographs of chloroplasts in M (C) and BS (D) cells of the WT and five GLK transgenic rice plants. Bars = 0.5 μ m. P, plastoglobuli; CW, cell wall. E, Averaged chloroplast numbers in each BS cell. The number of chloroplasts in each BS cell was determined from 15 BS cells in cross sections. F, Averaged thylakoid area per M chloroplast area. Thylakoid area per chloroplast area was determined from 6 to 8 M cells. Statistical tests were based on the Wilcoxon–Mann–Whitney U test for nonparametric comparison of two groups. The asterisk marked on a box indicates statistical significance in comparison with the WT ($*P < 0.05$; $**P < 0.01$). Boxplots showing the median (horizontal line) and interquartile range (boxes). Whiskers extend from the ends of the box to the smallest and largest data values (Adopted from Yeh et al., 2021)

4 Enhancing Photosynthesis Through Genetic Modification

4.1 Genetic engineering approaches targeting photosynthetic genes

When it comes to improving the photosynthetic efficiency of rice, everyone always thinks of genetic engineering. Indeed, it has now become a major means. However, there are still many ways to do it. For example, some studies have tried to make certain transcription factors "express more". For example, OsDREB1C of the DREB family, after this gene is expressed more in rice, not only photosynthesis is enhanced, but also nitrogen utilization efficiency is improved, and yield is also increased (Wei et al., 2022). Moreover, it affects more than one pathway - it can activate a series of genes related to photosynthesis, nitrogen metabolism and even flowering time. Overall, resource utilization is optimized and plant development becomes more favorable. On the other hand, it is not only "enhancement" that is effective. Sometimes, knocking out factors that have a negative effect on photosynthesis can also achieve good results. Like NRP1, this factor originally inhibits the expression of photosynthetic genes. After knocking it out, the photosynthetic capacity and biomass accumulation of rice have also been significantly improved (Chen et al., 2020). Therefore, whether it is "giving a push" or "pulling away an obstacle", it can help improve photosynthetic efficiency.

4.2 Integration of C₄-like traits into C₃ plants

C₃ plants such as rice always perform less than ideally in high temperature and drought environments, so scientists have been trying to learn from the "advantages" of C₄ plants. Corn, for example, is a typical C₄ crop. It has a natural advantage in reducing photorespiration, and therefore performs better in water and nitrogen utilization efficiency. However, it is not a simple matter to move the C₄ mechanism to rice. Some studies have tried to introduce several key enzymes in the C₄ pathway into rice, such as carbonic anhydrase (CA), PEPC and PPDK. What is the result? The genetically modified rice did show higher photosynthetic efficiency, and even increased its yield by about 12% (Sen et al., 2016). But the introduction of enzymes is only the first step, and there are more complex problems to be solved, such as the gene network that regulates these processes. In recent years, using genome editing technology and transcriptome analysis, researchers have also found some genes and regulatory factors that may help rice "transform" to the C₄ process (Karki et al., 2013; Wang et al., 2016b). Although this road has not yet been completely opened, the direction has become clearer and clearer.

4.3 Case studies on successful genetic modifications to enhance photosynthesis in rice

Not all genetic improvements will lead to the expected increase in yield, but some cases do have significant effects. For example, a study introduced the GOLDEN2-LIKE (GLK) gene from corn into rice, which resulted in an increase in chlorophyll content, enhanced light capture ability, and reduced light inhibition. In the end, the increase in rice biomass and grain yield was as high as 30%~40% (Li et al., 2020). Of course, this is just one approach. Other studies have started with non-coding RNA, such as overexpressing microRNA408 (miR408). This approach is also very effective in improving light energy utilization and CO₂ fixation, and field trials have also shown that miR408 can significantly increase yield, mainly because grains grow larger (Pan et al., 2018). In addition to these "input-type" modifications, some people have also tried to make more detailed changes, such as finely regulating the developmental path of chloroplasts in bundle sheath cells. Although this method is technically more complicated, it can further enhance the photosynthesis capacity and yield of rice (Lee et al., 2021). Therefore, sometimes changing the angle may have better results than expected.

5 Molecular Techniques for Photosynthetic Gene Manipulation

5.1 CRISPR/Cas9 and other genome editing tools

How popular is CRISPR/Cas9? Almost everyone who does genetic improvement has heard of it. The reason why this technology has been rapidly promoted is mainly because it is simple to operate, efficient, and quite accurate. In the field of rice, its usage is becoming more and more flexible. For example, in indica rice, a study used CRISPR/Cas9 to knock out a hexokinase gene called OsHXK1. The results showed that the light saturation point, stomatal conductance, and light tolerance were all increased, and photosynthetic products were also increased, and finally the yield naturally increased (Zheng et al., 2021). Of course, not only a single gene can be changed, CRISPR can also do "multi-gene linkage". For example, one study changed the yield-related genes Gn1a, DEP1,

GS3 and IPA1 at one time, and the number of panicles, panicle structure and grain size of rice were improved (Li et al., 2016). Some people have used this technology to make fragrant and high-yielding rice - they knocked in three P450 homologous genes plus OsBADH2 at the same time (Usman et al., 2020). In addition to yield, resistance can also be managed. For example, by editing the OsSAP gene related to drought response, the drought resistance of rice has also been improved (Park et al., 2022). In the final analysis, CRISPR/Cas9 brings a whole set of more flexible tools to breeding, rather than a single "universal improvement method."

5.2 RNA interference (RNAi) and gene silencing

The technology of RNA interference (RNAi) has actually been used in plant function research and crop improvement for a long time. What it is best at is "shutting up a gene" to see what happens if it is not expressed, or simply using it to inhibit genes that are not conducive to traits. In the field of photosynthesis, researchers often use RNAi to downregulate some genes that play a negative regulatory role, so that indicators such as photosynthetic rate and biomass accumulation are expected to increase. However, to be honest, there are not many RNAi studies specifically targeting rice photosynthesis-related genes, and the cases are not rich. But this does not mean that it is not applicable. On the contrary, judging from the practice of other crops, the potential of this technology is obvious to all, and it stands to reason that it can be used as a reference for rice. It's just that how to use it and where to use it still requires more attempts and verification.

5.3 Use of marker-assisted selection and molecular breeding

Molecular marker-assisted selection (MAS) has become more and more common in rice breeding in recent years, especially when used in combination with traditional breeding methods, the effect will be more obvious. In the past, selecting varieties based on experience took a long time and was inefficient; but with molecular markers, we have a better idea of which plants to keep and which to eliminate. For example, a study successfully introduced multiple QTL loci related to high yield into excellent rice varieties through MAS, and the yield increased significantly (Zhou et al., 2018). Of course, MAS alone is not a panacea. Now many breeding projects simply use it together with gene editing tools such as CRISPR/Cas9. The combination of the two sides can not only accurately locate, but also quickly change, and the overall efficiency is much higher. This approach does save a lot of time and effort for rice improvement that wants to improve photosynthetic efficiency and yield.

6 Impact of Photosynthetic Efficiency on Yield

6.1 Correlation between enhanced photosynthesis and increased biomass/yield

The increase in rice yield and biomass is often closely related to the improvement of photosynthesis efficiency. For example, a study found that after overexpressing the transcription factor OsDREB1C, the photosynthetic capacity of rice was significantly enhanced, and the yield of field trials increased by 41.3% to 68.3%. This improvement is mainly due to the increase in the number of grains per ear, the increase in grain weight, and the improvement of the harvest index (Wei et al., 2022). However, there is more than one similar effect. The continuous expression of the GOLDEN2-LIKE (GLK) gene of corn in rice can also increase chlorophyll content and light capture efficiency, resulting in an increase in biomass and grain yield by 30%~40% (Li et al., 2020). In addition, there are studies that have observed an increase in photosynthetic efficiency and biomass by knocking out a negative regulatory factor called NRP1, and these results have performed well in actual fields (Chen et al., 2020). It can be said that these ideas have enhanced the photosynthetic performance of rice from different angles.

6.2 Metabolic engineering for improved carbon assimilation

There have been many attempts to use metabolic engineering to improve rice's carbon assimilation capacity. For example, when the transcription factor mEmBP-1 of corn was overexpressed, multiple photosynthesis genes related to the Calvin-Benson-Bassham cycle were activated, resulting in an increase in rice photosynthetic rate, biomass, and grain yield (Perveen et al., 2020). However, this is not the only path. Overexpression of microRNA408 has also been shown to improve light utilization efficiency and carbon dioxide fixation capacity, and plant growth and yield have also improved accordingly (Pan et al., 2018). In addition, there are studies focusing on genes that affect carbon dioxide diffusion and assimilation, such as OsαCA1. Their identification and

regulation provide new evidence and ideas for metabolic engineering in increasing photosynthetic efficiency and yield (He et al., 2023). In the final analysis, metabolic engineering is not just about changing a few pathways, but also a strategy to improve photosynthetic capacity from multiple angles.

6.3 Physiological changes and yield improvement strategies

The increase in yield is inseparable from the various physiological changes brought about by genetic improvement, but the specific process is actually quite complicated. For example, after the transcription factor HYR is overexpressed, the photosynthesis of rice can be enhanced in various environments, and even under stress conditions such as drought and high temperature, the grain yield is maintained well (Ambavaram et al., 2014). However, this is not all. Factors that regulate photosynthesis and nitrogen utilization, such as OsDREB1C, show the potential to simultaneously improve yield and nitrogen utilization efficiency, and can also shorten the growth period of rice (Wei et al., 2022). Another detail is that some key components in the electron transport chain, such as the Rieske FeS protein in the cytochrome b6/f complex, can also affect plant growth and grain yield. This shows that starting from the photosynthetic efficiency of leaves and controlling certain key steps is actually an effective way to increase yield (Yamori et al., 2016). These improvement strategies from different angles have made people more confident about the path to increase rice yield.

7 Environmental Influences on Photosynthesis and Gene Expression

7.1 Role of light, temperature, and water availability

Rice photosynthesis is actually quite affected by the environment. Let's talk about light first. After all, how can photosynthesis occur without light? But what's interesting is that light not only provides energy, but also affects gene expression. For example, the transcription factor OsDREB1C, once stimulated by light, the photosynthetic capacity increases, and the grain yield and nitrogen fertilizer utilization also improve (Wei et al., 2022). Temperature is also a key factor. Generally speaking, high temperature is not a good thing for rice and can easily cause stress. But then again, some genes are quite resistant - transcription factors like HYR can stabilize photosynthesis under high temperatures, so that the yield does not drop too much (Ambavaram et al., 2014). As for water, let alone. During drought, photosynthetic efficiency will definitely be affected. But rice is not completely helpless. Genes like OsSAP16 can regulate stress responses and at least improve water use efficiency (Wang et al., 2016a). So you see, once environmental conditions change, rice's photosynthesis and gene expression have to be adjusted accordingly. It's not that simple.

7.2 Stress-responsive genes and their impact on photosynthetic efficiency

When rice encounters drought, high temperature and other bad things, its photosynthetic efficiency is easy to drop. But what's interesting is that some genes are specifically responsible for "saving the day". For example, the transcription factor HYR can directly activate photosynthesis-related genes, and also mobilize carbon metabolism genes (Ambavaram et al., 2014). In this way, even if the weather is not good, photosynthesis can be barely maintained. When it comes to coping with stress, OsDREB1C is also a tough guy. It controls a large group of genes, which can not only improve photosynthetic capacity but also improve nitrogen fertilizer utilization efficiency (Wei et al., 2022). This is particularly important in harsh environments. After all, whether the yield can be maintained depends on these. There is also an interesting discovery. The GLK gene of corn was overexpressed in rice, and the result was quite unexpected-not only did it use light energy better, but it also reduced light inhibition (Li et al., 2020). These genes are like the "emergency system" of plants. No matter how bad the environment is, photosynthesis can continue to work, so that the yield is not too bad.

7.3 Adaptive strategies for climate change resilience

It is not easy to grow rice nowadays, as climate change is making the weather more extreme. However, rice has its own ways to cope with the situation, such as adjusting its photosynthesis and stress response genes. Take the OsDREB1C gene for example. Studies have found that overexpressing it has a good effect - not only can it enhance photosynthesis, but it can also make nitrogen fertilizer use more efficient, and even better, it can make rice bloom earlier (Wei et al., 2022). In this way, resource allocation is more reasonable, and yield will naturally

increase. Speaking of drought resistance, the OsSAP16 gene is also very interesting. It mainly does two things: improve water use efficiency and regulate a large number of stress response genes (Wang et al., 2016a). When drought comes, these genes are like emergency teams that can help rice survive. Recently, scientists have also developed an analysis method called EGRINs (Wilkins et al., 2016). This thing is powerful because it can sort out the complex relationship between gene expression under environmental stress, which is very helpful for breeding rice varieties that are resistant to climate change. But then again, how these discoveries can be used to improve varieties will depend on follow-up research.

8 Case Study: Enhancing Yield through Photosynthetic Gene Regulation

8.1 Background and context of the case study

When it comes to rice (*Oryza sativa*), many people's first reaction is that it feeds billions of people around the world. But you will also find that although traditional breeding has pushed up yields all the way, it may not be able to meet the new demands brought about by the rapid population growth and changes in dietary structure. For this reason, genetic engineering has begun to attract much attention-especially whether it can further improve photosynthetic efficiency to achieve higher yields. This case study does not follow the old routine, but focuses on the genetic elements that regulate photosynthesis, especially the manipulation of transcription factors and other key factors, to see how to increase rice production and use resources more efficiently.

8.2 Methods used for improving photosynthetic efficiency in the selected rice cultivar

When doing genetic modification, researchers have tried several ideas, and the effects of different methods are also different. Let me first give you an interesting example: someone "moved" the mEmBP-1 of corn into rice, and as a result, all the genes related to photosynthesis became agitated, with more chlorophyll, soaring photosynthetic rate, and increasing biomass and grain yield (Perveen et al., 2020). Let's talk about OsDREB1C. After overexpression in some varieties, grain yield can jump to 41.3%~68.3%, nitrogen utilization efficiency is not lowered, and even the growth period can be slightly compressed-however, this range will fluctuate in different environments (Wei et al., 2022). Finally, HYR can be regarded as a universal key. Whether it is drought, high temperature or normal environment, photosynthesis can be enhanced, yield is steadily increased, and drought and heat resistance are also rising (Ambavaram et al., 2014). These examples tell us that if we want to achieve higher photosynthetic efficiency, we cannot rely on just one method, but must flexibly choose strategies based on conditions.

In genetic improvement, natural variation is also an option. For example, the gene NAL1 (also known as GPS) can increase the number of mesophyll cells and thicker leaves, increase the photosynthetic rate and grain yield, and the plant morphology is not affected (Takai et al., 2013). When it comes to CRISPR/Cas9, it is more flexible - after knocking out OsHXK1, the light saturation point, stomatal conductance and photosynthetic products are all increased, and the yield naturally increases (Ambavaram et al., 2014). There is another idea: remove those "obstacles". NRP1 originally inhibits photosynthetic genes. After CRISPR was used to knock it out, photosynthesis and biomass increased significantly (Figure 3) (Chen et al., 2020).

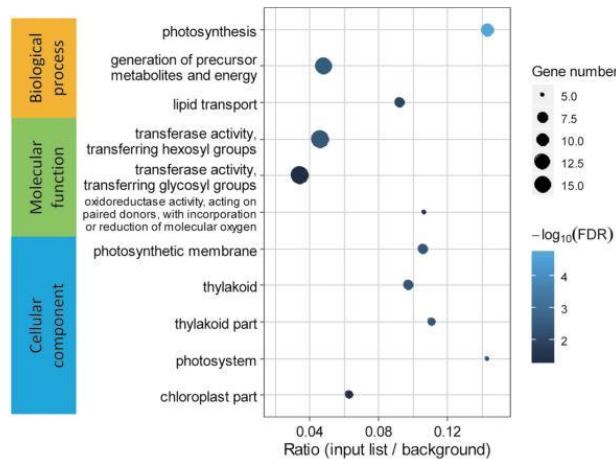
8.3 Outcomes, challenges, and implications for future breeding programs

These genetic improvements have indeed increased rice yields in field trials - for OsDREB1C and mEmBP-1, the yield increase ranges from 30% to more than 68% (Perveen et al., 2020; Wei et al., 2022). At the same time, higher chlorophyll content, enhanced light capture efficiency, and stronger carbon fixation capacity are all intuitive manifestations of improved photosynthetic performance (Li et al., 2020; Perveen et al., 2020). Interestingly, in the case of less fertilizer and insufficient nitrogen, these improvements also help rice to better utilize limited nitrogen sources and more rationally allocate resources, doing a lot of "good things" for sustainable agriculture (Wei et al., 2022).

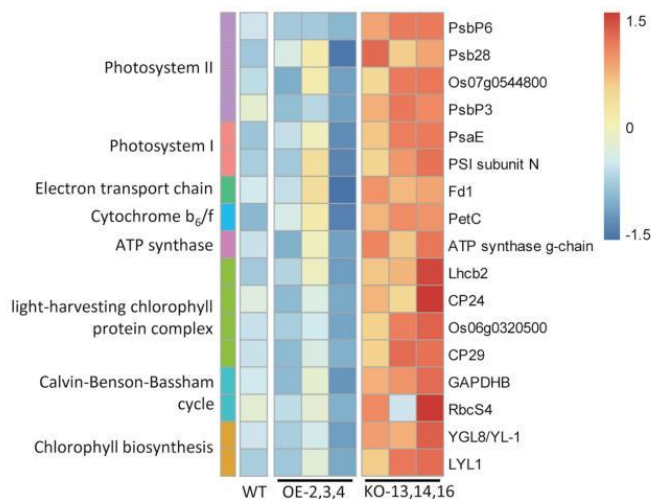
However, rice is not omnipotent. For example, HYR performs relatively well under stresses such as high temperature and drought, but not all improved genes can withstand the test of various field environments, and they need to be repeatedly verified under a wider range of conditions (Ambavaram et al., 2014). Furthermore, do not

forget the dual pressures of supervision and public opinion: whether genetically modified rice can be planted on a large scale and how to plant it depends on policies and public acceptance, which will add an extra obstacle to the promotion of technology.

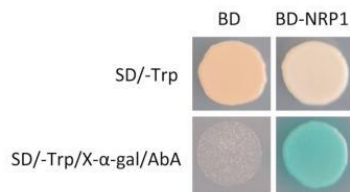
A



B



C



37

Figure 3 NRP1 can regulate the expression of photosynthetic genes (Adopted from Chen et al., 2020)

Image caption: (A) GO enrichment analysis of up-regulated genes in KO lines. Only top five enriched GO terms are shown if enriched GO terms are more than five. Ratio (input list/background) represents the ratio of gene numbers in the input list divided by background list in each GO term. (B) Heatmap of reproducible up-regulated genes of the photosynthetic pathway in KO lines. The values shown in the heatmap are transformed gene expression values for each line. During the data transformation, the gene expression values in each biological replicate were first normalized as transcript per million (TPM), then the mean of three biological replicates was calculated to obtain the gene expression value for each line. The average TPM was finally Z-score transformed as used in the heatmap. The symbols of genes in this heatmap are from the Rice Annotation Project (RAP) Database (<https://rapdb.dna.affrc.go.jp/>). The samples for RNA-seq were collected in field at the T3 generation in 2017. (C) Transactivation assay of NRP1. BD is pGBKT7 empty vector, which was used as a negative control in this assay. We fused NRP1 with the GAL4 DNA binding domain within pGBKT7 plasmid (BD-NRP1) and transformed this into Y2HGold yeast competent cells. Positive yeast clones which grew on the SD/-Trp medium were transferred to SD/-Trp/X-α-gal/AbA medium for assaying the transactivation (Adopted from Chen et al., 2020)

Looking at the patchwork of rice seedlings in the fields, you will understand that it is easy to go astray if you only rely on increasing yield as a goal. Future breeding will have to bundle multiple advantages together - enhancing photosynthesis, using nitrogen more intelligently, and improving drought and high temperature resistance. Only in this way can new rice varieties with both high and stable yields be cultivated. When it comes to precise gene modification, CRISPR/Cas9 is now an indispensable tool in the toolbox. It allows us to "operate" on specific genes and customize rice that is more suitable for different planting environments (Zheng et al., 2021). Thinking a step further, if these improvements can run on the track of improving photosynthetic efficiency and resource utilization at the same time, not only will farmers use less fertilizer, but they can also reduce the burden on the environment and move one step closer to sustainable agriculture.

9 Challenges and Limitations in Photosynthetic Gene Regulation

9.1 Complexity of genetic pathways and regulatory networks

When it comes to regulating photosynthetic genes in rice, it is not a matter of a few straight lines, but a complex genetic network. Transcription factors such as OsDREB1C, NRP1, and GLK must first find "exclusive seats" on DNA before they can adjust the gene expression of photosynthesis, nitrogen metabolism, and even other traits (Chen et al., 2020; Li et al., 2020; Wei et al., 2022). However, don't think that expressing one more OsDREB1C will simply bring benefits. It also involves a large number of photosynthesis and nitrogen nutrition-related genes. If it is over-expressed, it will be difficult to control (Wei et al., 2022). Similarly, if the NRP1 or GLK genes are activated, several downstream targets can be hit at once, which makes it difficult for us to fully understand how they affect photosynthesis (Chen et al., 2020; Li et al., 2020).

9.2 Trade-offs between photosynthetic efficiency and other agronomic traits

It seems that higher photosynthetic efficiency is not always a "purely cost-effective" deal. Sometimes you have to pay attention to other agronomic traits. Let's start with OsDREB1C. Overexpression can deliberately increase both yield and nitrogen use efficiency, but it can also bring about an earlier flowering period, which may be a problem in some planting seasons (Wei et al., 2022). Then there is NRP1. After knocking it out, photosynthesis and biomass soared, but at the same time, its impact on other physiological processes has not been fully understood, and sometimes it leads to new problems (Chen et al., 2020). Finally, the GLK gene of maize has been highly expressed in rice. Although it can improve photosynthesis and yield, it may also cause photoinhibition under certain light conditions (Li et al., 2020). These cases remind us that when doing genetic improvement, we must place the improvement of photosynthetic efficiency and the balance of other key traits on an equal footing.

9.3 Ethical, economic, and regulatory concerns in genetic modification

When using genetic modification to improve the photosynthetic efficiency of rice, the discussion is not as simple as "doubling the yield". Let's talk about the ethical aspect first: people have both expectations and concerns about genetically modified organisms (GMOs), especially whether they will have long-term impacts on ecosystems and species diversity. Looking at the economic aspect, developing a genetically modified rice variety, running field trials, and getting it on the market requires not only technical investment, but also huge costs and many links, and consumers' acceptance of such products is also a major variable. Not to mention that the approval processes in different countries and regions vary greatly, with some places progressing quickly and others being stuck. For example, even if the overexpression of transcription factors such as OsDREB1C can significantly increase yields and improve resource utilization in experiments, when it comes to large-scale promotion, it may be "dragged down" by regulatory barriers and public concerns (Chen et al., 2020; Li et al., 2020; Wei et al., 2022). To break the deadlock, comprehensive measures must be adopted: from rigorous safety assessments, transparent stakeholder dialogues, to a policy framework that strikes a balance between innovation and risk prevention, all of which are indispensable.

10 Future Directions and Recommendations

10.1 Integration of multi-omics approaches (genomics, transcriptomics, proteomics)

If you want to truly understand the regulatory mechanism behind rice photosynthesis, a single method is often not

enough. Now many laboratories have begun to put genomics, transcriptomics and proteomics into the same pot, trying to capture changes at different levels. For example, RNA sequencing (RNA-seq) data helped us lock in the transcription factor NRP1 that "quietly" inhibits photosynthesis-if it were not combined with multi-omics verification, it would probably take a lot of effort to find it (Chen et al., 2020). Looking further down, transcriptome and proteome analysis revealed the important role of OsDREB1C more clearly: it not only improves photosynthetic efficiency, but also helps nitrogen utilization (Wei et al., 2022). However, these omics data sometimes "fight" with each other, so integrated analysis is particularly critical. Using all these methods can not only dig up more new genes and regulatory elements, but also provide more precise targets for improving high-yield and high-efficiency rice.

10.2 Combining traditional breeding and molecular techniques for enhanced photosynthesis

There are two competing ideas on the road - one is natural variation screened out by field trials for many years, and the other is to directly "modify genes". Let's talk about traditional breeding first. Natural variants like NAL1 are typical representatives. They can be used right away and can significantly improve photosynthetic capacity and yield (Takai et al., 2013). It saves trouble, but the effect is slow. Therefore, molecular technologies such as CRISPR/Cas9 were brought in - after knocking out OsHXX1, the photosynthetic rate and yield of rice increased steadily (Zheng et al., 2021). However, it is more interesting not to separate these two paths: overexpressing the transcription factor mEmBP-1 and GLK together combines the accumulated advantages of traditional breeding with the precision and efficiency of modern molecular methods, resulting in significant improvements in photosynthesis and yield (Li et al., 2020; Perveen et al., 2020). This way, the speed of developing high-yield rice is different.

10.3 Policy and collaborative efforts for sustainable yield improvement

If we want to steadily increase rice yields, technology alone is not enough, policies and cooperation are equally critical. Financial support often cannot keep up with the pace, and sometimes it takes a long time to get government funding, but once the public and private sectors join forces, they can push the results of genetic improvement in the laboratory to the field. Take the varieties that express OsDREB1C as an example. Although the research and development process was tortuous, field trials and large-scale promotion showed significant increases in yield and nitrogen use efficiency (Wei et al., 2022). In addition, international collaboration is also essential: countries share knowledge and integrate resources so that the latest research can truly serve global food security. Ultimately, only with policy support, funding follow-up, and cross-border cooperation can new technologies be implemented faster and promote sustainable agricultural development.

Acknowledgments

We would like to thank the comments from two anonymous peer reviewers on the manuscript of this study.

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.

References

- Adachi S., Ohkubo S., San N., and Yamamoto T., 2020, Genetic determination for source capacity to support breeding of high-yielding rice (*Oryza sativa*), *Molecular Breeding*, 40: 20.
<https://doi.org/10.1007/s11032-020-1101-5>
- Ambavaram M., Basu S., Krishnan A., Ramegowda V., Batlang U., Rahman L., Baisakh N., and Pereira A., 2014, Coordinated regulation of photosynthesis in rice increases yield and tolerance to environmental stress, *Nature Communications*, 5: 5302.
<https://doi.org/10.1038/ncomms6302>
- Chen F., Zheng G., Qu M., Wang Y., Lyu M., and Zhu X., 2020, Knocking out a *NEGATIVE REGULATOR OF PHOTOSYNTHESIS 1 (NRP1)* increases rice leaf photosynthesis and biomass production in the field, *Journal of Experimental Botany*, 72(5): 1836-1849.
<https://doi.org/10.1093/jxb/eraa566>
- Cohen S., and Leach J., 2019, Abiotic and biotic stresses induce a core transcriptome response in rice, *Scientific Reports*, 9: 6273.
<https://doi.org/10.1038/s41598-019-42731-8>

- 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>
- Ermakova M., Danila F., Furbank R., and Von Caemmerer S., 2019, On the road to C₄ rice: advances and perspectives, *The Plant Journal*, 101: 940-950.
<https://doi.org/10.1111/tpj.14562>
- He Y., Duan W., Xue B., Cong X., Sun P., Hou X., and Liang Y., 2023, OsaCA1 affects photosynthesis, yield potential, and water use efficiency in rice, *International Journal of Molecular Sciences*, 24(6): 5560.
<https://doi.org/10.3390/ijms24065560>
- Huang Y.M., 2024, Cultural weed management strategies in rice cultivation: reducing the infestation of weedy rice, *Field Crop*, 7(2): 105-115.
<https://doi.org/10.5376/fc.2024.07.0011>
- Karki S., Rizal G., and Quick W., 2013, Improvement of photosynthesis in rice (*Oryza sativa* L.) by inserting the C₄ pathway, *Rice*, 6: 28.
<https://doi.org/10.1186/1939-8433-6-28>
- Lee D., Hua L., Khoshraveh R., Giuliani R., Kumar I., Cousins A., Sage T., Hibberd J., and Brutnell T., 2021, Engineering chloroplast development in rice through cell - specific control of endogenous genetic circuits, *Plant Biotechnology Journal*, 19: 2291-2303.
<https://doi.org/10.1111/pbi.13660>
- Li M., Li X., Zhou Z., Wu P., Fang M., Pan X., Lin Q., Luo W., Wu G., and Li H., 2016, Reassessment of the four yield-related genes *Gn1a*, *DEP1*, *GS3*, and *IPA1* in rice using a CRISPR/Cas9 system, *Frontiers in Plant Science*, 7: 377.
<https://doi.org/10.3389/fpls.2016.00377>
- Li X., Wang P., Li J., Wei S., Yan Y., Yang J., Zhao M., Langdale J., and Zhou W., 2020, Maize *GOLDEN2-LIKE* genes enhance biomass and grain yields in rice by improving photosynthesis and reducing photoinhibition, *Communications Biology*, 3: 151.
<https://doi.org/10.1038/s42003-020-0887-3>
- Pan J., Huang D., Guo Z., Kuang Z., Zhang H., Xie X., Ma Z., Gao S., Lerdau M., Chu C., and Li L., 2018, Overexpression of microRNA408 enhances photosynthesis, growth, and seed yield in diverse plants, *Journal of Integrative Plant Biology*, 60(4): 323-340.
<https://doi.org/10.1111/jipb.12634>
- Park J., Kim E., Jang Y., Jan R., Farooq M., Ubaidillah M., and Kim K., 2022, Applications of CRISPR/Cas9 as new strategies for short breeding to drought gene in rice, *Frontiers in Plant Science*, 13: 850441.
<https://doi.org/10.3389/fpls.2022.850441>
- Paul M., 2021, Improving photosynthetic metabolism for crop yields: what is going to work? *Frontiers in Plant Science*, 12: 743862
<https://doi.org/10.3389/fpls.2021.743862>
- Perveen S., Qu M., Chen F., Essemine J., Khan N., Lv M., Chang T., Song Q., Chen G., and Zhu X., 2020, Over-expression of maize transcription factor mEMB1 increases photosynthesis, biomass and yield in rice, *Journal of Experimental Botany*, 71(16): 4944-4957.
<https://doi.org/10.1093/jxb/eraa248>
- Sen P., Ghosh S., Sarkar S., Chanda P., Mukherjee A., Datta S., and Datta K., 2016, Pyramiding of three C₄ specific genes towards yield enhancement in rice, *Plant Cell, Tissue and Organ Culture (PCTOC)*, 128: 145-160.
<https://doi.org/10.1007/s11240-016-1094-2>
- Takai T., Adachi S., Taguchi-Shiobara F., Sanoh-Arai Y., Iwasawa N., Yoshinaga S., Hirose S., Taniguchi Y., Yamanouchi U., Wu J., Matsumoto T., Sugimoto K., Kondo K., Ikka T., Ando T., Kono I., Ito S., Shomura A., Ookawa T., Hirasawa T., Yano M., Kondo M., and Yamamoto T., 2013, A natural variant of *NAL1*, selected in high-yield rice breeding programs, pleiotropically increases photosynthesis rate, *Scientific Reports*, 3: 2149.
<https://doi.org/10.1038/srep02149>
- Usman B., Nawaz G., Zhao N., Liu Y., and Li R., 2020, Generation of high yielding and fragrant rice (*Oryza sativa* L.) lines by CRISPR/Cas9 targeted mutagenesis of three homoeologs of cytochrome P450 gene family and *OsBADH2* and transcriptome and proteome profiling of revealed changes triggered by mutations, *Plants*, 9(6): 788.
<https://doi.org/10.3390/plants9060788>
- Wang F., Coe R., Karki S., Wanchana S., Thakur V., Henry A., Lin H., Huang J., Peng S., and Quick W., 2016a, Overexpression of *OsSAP16* regulates photosynthesis and the expression of a broad range of stress response genes in rice (*Oryza sativa* L.), *PLoS ONE*, 11(6): e0157244.
<https://doi.org/10.1371/journal.pone.0157244>
- Wang P., Vlad D., and Langdale J., 2016b, Finding the genes to build C₄ rice, *Current Opinion in Plant Biology*, 31: 44-50.
<https://doi.org/10.1016/j.cpb.2016.03.012>
- Wei S., Li X., Lu Z., Zhang H., Ye X., Zhou Y., Li J., Yan Y., Pei H., Duan F., Wang D., Chen S., Wang P., Zhang C., Shang L., Zhou Y., Yan P., Zhao M., Huang J., Bock R., Qian Q., and Zhou W., 2022, A transcriptional regulator that boosts grain yields and shortens the growth duration of rice, *Science*, 377: 6604.
<https://doi.org/10.1126/science.abi8455>
- Wilkins O., Hafemeister C., Plessis A., Holloway-Phillips M., Pham G., Nicotra A., Gregorio G., Jagadish S., Septiningsih E., Bonneau R., and Purugganan M., 2016, EGRINs (environmental gene regulatory influence networks) in rice that function in the response to water deficit, high temperature, and agricultural environments, *Plant Cell*, 28: 2365-2384.
<https://doi.org/10.1105/tpc.16.00158>

- Yamori W., Kondo E., Sugiura D., Terashima I., Suzuki Y., and Makino A., 2016, Enhanced leaf photosynthesis as a target to increase grain yield: insights from transgenic rice lines with variable Rieske FeS protein content in the cytochrome b6 /f complex, *Plant, Cell & Environment*, 39(1): 80-87.
<https://doi.org/10.1111/pce.12594>
- Yeh S., Lin H., Chang Y., Chang Y., Chang C., Huang Y., Ho Y., Lin C., Zheng J., Jane W., Ng C., Lu M., Lai I., To K., Li W., and Ku M., 2021, Maize Golden2-like transcription factors boost rice chloroplast development, photosynthesis, and grain yield, *Plant Physiology*, 188: 442-459.
<https://doi.org/10.1093/plphys/kiab511>
- Zhang X., Tang M., Wang H., Tao W., Wang Q., Chen L., Gao G., Lv R., and Liang T., 2022, Analysis of photosynthetic differences of rice germplasm in southeast Asia based on leaf-tissue structure, physiology, and iTRAQ, *Agronomy*, 12(12): 3207.
<https://doi.org/10.3390/agronomy12123207>
- Zhang Y., Zhou Y., Sun Q., Deng D., Liu H., Chen S., and Yin Z., 2019, Genetic determinants controlling maize rubisco activase gene expression and a comparison with rice counterparts, *BMC Plant Biology*, 19: 351.
<https://doi.org/10.1186/s12870-019-1965-x>
- Zheng S., Ye C., Lu J., Liufu J., Lin L., Dong Z., Li J., and Zhuang C., 2021, Improving the rice photosynthetic efficiency and yield by editing *OsHXX1* via CRISPR/Cas9 system, *International Journal of Molecular Sciences*, 22(17): 9554.
<https://doi.org/10.3390/ijms22179554>
- Zhou J., Xin X., He Y., Chen H., Li Q., Tang X., Zhong Z., Deng K., Zheng X., Akher S., Cai G., Qi Y., and Zhang Y., 2018, Multiplex QTL editing of grain-related genes improves yield in elite rice varieties, *Plant Cell Reports*, 38: 475-485.
<https://doi.org/10.1007/s00299-018-2340-3>
- Zhou L., Xiang X., Ji D., Chen Q., Ma T., Wang J., and Liu C., 2023, A carbonic anhydrase, ZmCA4, contributes to photosynthetic efficiency, and modulates CO₂ signaling gene expression by interacting with aquaporin ZmPIP2;6 in maize, *Plant & Cell Physiology*, 65(2): 243-258.
<https://doi.org/10.1093/pcp/pcad145>



Disclaimer/Publisher's Note

The statements, opinions, and data contained in all publications are solely those of the individual authors and contributors and do not represent the views of the publishing house and/or its editors. The publisher and/or its editors disclaim all responsibility for any harm or damage to persons or property that may result from the application of ideas, methods, instructions, or products discussed in the content. Publisher remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.
