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How Rice Senses and Adapts to Changes in Temperature and Light

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Abstract Rice (*Oryza sativa* L.) is an important food crop, but its production faces severe challenges due to climate change, especially fluctuations in temperature and light. This study explores the mechanisms by which rice senses and adapts to these environmental changes. High temperatures adversely affect rice growth and yield, so understanding the mechanisms of heat tolerance is of great significance. Studies have identified key genes and pathways involving auxin and abscisic acid (ABA), which play an important role in rice's response to heat stress. In addition, RNA modifications such as 5-methylcytosine (m5C) improve rice's ability to adapt to high temperatures by stabilizing photosynthesis and detoxification systems. The interaction between light and circadian rhythms also significantly affects rice's response to temperature stress, and certain specific genes improve rice's cold tolerance by coordinating these responses. This study integrates current research on the molecular, genetic, and physiological responses of rice to temperature and light stress, and provides potential breeding strategies for cultivating rice varieties with strong stress resistance.

Keywords Rice (*Oryza sativa* L.); Temperature stress; Light adaptation; Thermotolerance; Circadian clock

1 Introduction

When it comes to the growth of rice (*Oryza sativa* L.), many people know that temperature and light are important, but how important are they? Let's talk about light first. This thing not only affects photosynthesis, but also affects the energy balance of the entire plant. But what's interesting is that too much light or too long duration is not necessarily a good thing (Li et al., 2022; Oh et al., 2023; Ren et al., 2023). This is even more true for temperature. Although suitable temperature can indeed promote basic physiological activities such as photosynthesis and respiration, once the temperature is too high, trouble will come-the plant will suffer irreversible damage, especially when flowering and heading, which directly affects the quality of rice. Of course, if the temperature and light are not in the appropriate range, the problem will be more obvious: slow growth, low yield, and poor rice quality are all common situations. But then again, different varieties of rice may respond differently to these conditions.

It is becoming increasingly difficult to grow rice now - the weather changes at any time, and high temperatures often occur. You may not know that this extreme high temperature affects rice growth from the seedling stage to the heading stage, and the yield naturally decreases (Rabara et al., 2020; Sharma et al., 2021; Li et al., 2022). Moreover, this is not the end. Experts say that this kind of weather will only become more and more frequent in the future. The most troublesome thing is that high temperatures often come with drought. How can rice withstand this? So now breeders are keeping an eye on this matter and trying every means to cultivate more resistant varieties. But then again, even if you have a good variety, the harvest will still be discounted in a year of continuous high temperature and drought (Fahad et al., 2019).

Anyone who studies rice knows that climate change is causing more and more trouble. Our main goal in this study is to understand how rice senses and adapts to changes in temperature and light - from the most basic molecular reactions to the physiological changes of the entire plant. But to be honest, this is not that simple, after all, it involves complex processes such as genetic regulation and signal transduction (Rabara et al., 2020; Sharma et al., 2021). Of course, the most important thing is to find varieties that are truly resistant to high temperatures and strong light, which is good news for breeders. Research in this area has progressed rapidly in recent years, but it has also encountered many difficulties. We have sorted out all of this and hope to provide some reference for scientific researchers, farmers, and policy makers. After all, under the current climate conditions, if we want to grow good rice stably, we really need everyone to work together to find a solution.

2 Mechanisms of Temperature Sensing in Rice

2.1 Molecular pathways involved in temperature perception

Rice is an interesting crop. When it encounters high temperatures, it actually has its own way of dealing with it. For example, studies have found that the transcription factor OsHSFA2d changes its form under high temperatures and becomes an active form, OsHSFA2dI (Cheng et al., 2015). This thing is very important. It can help regulate the expression of some important genes and keep the proteins in the cell stable. Of course, this is not all. Genes such as OsIAA13 and OsIAA20 will also join in the fun and be particularly active at high temperatures (Sharma et al., 2021). Recently, there is also a new discovery that the G-quadruplex structure in the promoter of some genes can sense temperature changes (Chang et al., 2022). But then again, although rice has these defense mechanisms, if it really encounters sustained high temperatures, these protective measures may not be enough.

When rice encounters low temperatures, it is actually more "smart" than we thought. It activates a whole set of defense systems from the cell membrane to the cell nucleus, just like transmitting alarms layer by layer. The first to move is the calcium ion signal, followed by various protein kinases. Interestingly, the CBF/DREB pathway is particularly critical (Guo et al., 2018), which can command many cold-resistant genes to start working. However, the most surprising thing is that a large amount of hydrogen peroxide will suddenly be produced in the cells at low temperatures, which instead becomes a distress signal and activates transcription factors such as bZIP and ERF. Just like our adrenaline surges when we encounter danger, rice will also initiate this emergency response. Another interesting phenomenon is that rice's cold resistance is actually related to its biological clock (Lu et al., 2020), although scientists are still studying how it works. To be honest, if I hadn't seen these research data with my own eyes, it would be hard to imagine that a rice plant can have so many tricks to deal with the cold.

2.2 Physiological responses to temperature extremes

Rice is an interesting crop. It can always come up with various ways to deal with extreme temperatures. Let’s talk about high temperatures first. At this time, the cell membrane will become particularly "flexible", just like applying a layer of sunscreen to itself (Kan and Lin, 2021). A cleaning system will also be activated in the cell to deal with harmful free radicals. The most amazing thing is those heat shock proteins, which are like "first aid" for proteins, helping other proteins to maintain their normal form (Qiu et al., 2023). The situation is different at low temperatures. Rice will make some "antifreeze" by itself and readjust gene expression. However, there is a contradiction here: it is necessary to ensure normal growth while enhancing cold resistance (Guo et al., 2018). Just like people need to stay active and keep warm in winter, this balance is quite difficult to grasp. Interestingly, the same temperature change may cause rice to react completely differently at different growth stages.

2.3 Key genes and transcription factors associated with temperature sensing

Rice is an interesting crop. When faced with temperature changes, it is like there is a "command center" in its body that dispatches troops. Let's talk about high temperature first. Genes like OsHSFA2d are particularly busy. They have to deal with proteins that are damaged by heat (Cheng et al., 2015). There is also a guy called OsbZIP14 that is even more interesting. It works with OsbZIP58 to specifically manage protein storage in seeds at high temperatures (Qiu et al., 2023). The situation is different at low temperatures. The CBF/DREB system is like a master switch (Guo et al., 2018), which activates various defense programs when encountering low temperatures. The most amazing are transcription factors such as bZIP and ERF, which are particularly sensitive to oxidative signals and take action immediately at the slightest sign of trouble. But to be honest, how these systems work together is still a mystery.

3 Mechanisms of Light Sensing in Rice

3.1 Photoreceptors in rice: structure and function

Sometimes, you will see the "light game" between rice plants in the field - in fact, all this is inseparable from the receptor of phytochrome. First of all, let me give you a background: plants rely on it to "listen" to the "sounds" of red light and far-red light, which directly affects the growth rhythm of rice. For example, phytochrome B (phyB), this guy is best at sensing the ratio of red light to far-red light (R\:FR ratio). However, it should be noted that not every time the R\:FR ratio decreases, the stem must be elongated - some varieties are slow to respond to shade, but in most cases, they will accelerate stem elongation by producing more auxin and gibberellin (Pierik and Ballaré, 2020; Ma, 2024). In addition, this guy can also intervene in the defense system and adjust the jasmonic acid signaling pathway so that plants can have more tricks when facing pathogens. When it comes to photoperiod control of flowering, phytochrome A (phyA) and B are not always on the same side: sometimes they go their own way, and sometimes they come together to cooperate, depending on how the light conditions are.

Sometimes you may think that the "blue light switches" in the leaves are really not simple - cryptochromes and phytochromes are actually blue light receptors, the purpose of which is to make photosynthesis more efficient and various light reactions more flexible. Let's talk about cryptochromes (cry1 and cry2): they help seedlings gain a foothold, calibrate circadian rhythms, and can also drive plants from the "eating nutrients" stage to the "flowering and fruiting" stage. However, there are a few strains that are not so dependent on them. All of this is achieved by them working in concert with other photoreceptors and signal molecules. As for phytochromes, don't think that it only controls red light-it can also sense blue light, control phototropism, light-induced stomatal opening and chloroplast swinging, which is the key to truly turning light energy into nutrients. After phytochromes receive photons, they will change their "body" and activate their own protein kinases, and then a series of signals will unfold.

3.2 Signal transduction pathways regulating light responses

Sometimes you might think that the signaling pathways in rice are like a complex social network - light receptors and transcription factors are pieced together and influence each other. But don't forget that not all links are perfect, and some transcription factors respond differently in different varieties. Let's talk about the most conspicuous ones first: phytochromes, cryptochromes, and phytochromes, once they receive light signals, they will set off a series of cascade reactions, reorganize the transcription program, and mark some signaling proteins for degradation. By the way, the COP1/SPA ubiquitin E3 ligase complex and PIF transcription factors are key hubs, and their activity is directly dragged around by light receptors (Pierik and Ballaré, 2020). Another detail - under light, phytochromes and UVR8 have to move to the nucleus to gather, and cryptochrome cry2 also has to accumulate in the nucleus. This step is crucial for activating light-responsive genes (Figure 1) (Jing and Lin, 2020). However, the most interesting thing is that this system is ultimately designed to allow rice plants to flexibly adjust their growth and development according to the surrounding light.

3.3 Influence of photoperiod on rice flowering and yield

The flowering time and yield of rice are actually closely related to the length of day and night (i.e., photoperiod). However, it is not that simple. Rice is a plant that flowers only when the daylight is short (short-day plant). Speaking of this, we have to mention phytochromes, especially phyA and phyB, which are in sync with the biological clock and can control flowering genes, and are particularly important in sensing photoperiods. Interestingly, the Hd1 gene (a relative of the Arabidopsis CO gene in rice) follows the rhythm of the biological clock to regulate the expression of FT homologous genes, which is the key to determining when to flower (Su et al., 2016). Moreover, there is a blue light response protein called OsHAL3, which can work with Hd1 to adjust the flowering time under different light conditions. You see, the entire network of rice's light sensing and signal transmission is indeed quite complex.

4 Integration of Temperature and Light Signaling in Rice

4.1 Cross-talk between temperature and light signaling pathways

Sometimes you feel that temperature and light signals in rice plants are like two old friends who are reluctant to sit together - they always have to find various excuses to chat. First of all, phytochrome B (phyB) is not only concerned with light, but also takes care of temperature. Once it is busy, it will pass information to the regulatory bosses below, such as phytochrome interacting factor 4 (PIF4) and constitutive photomorphogenic protein 1

(COP1) (Nohales et al., 2019). However, don't think that they have a smooth journey: COP1 is willing to help PIF4, but PIF4 itself has to listen to the orders of the circadian rhythm component early flowering 3 (ELF3) (Legris et al., 2017). More interestingly, reactive oxygen species (ROS) often "make trouble" nearby - it is not only connected with rhythm and calcium signals in series, but also plays an important role in light-temperature interaction (Krasensky-Wrzaczek and Kangasjärvi, 2018).

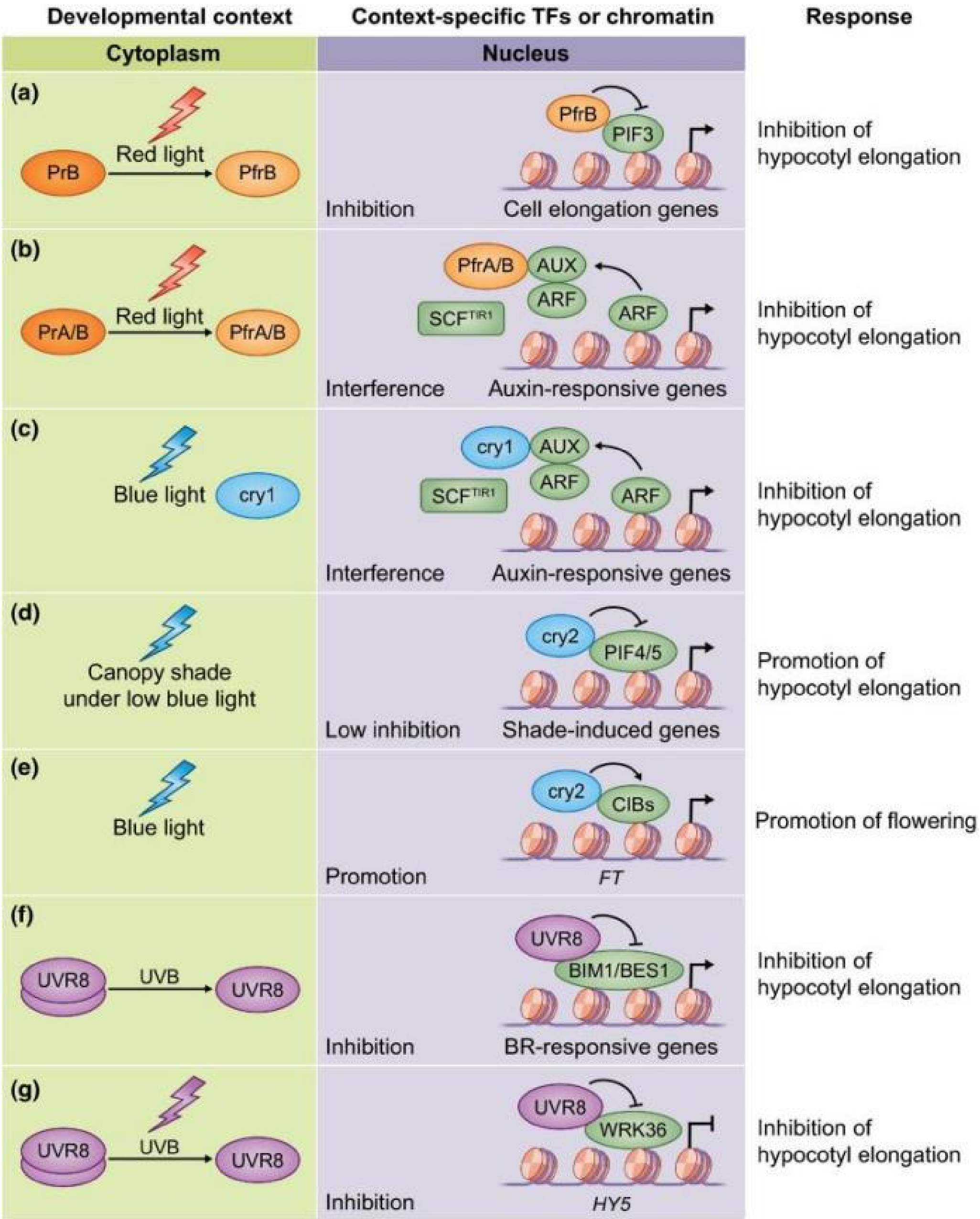

Figure 1 The representative signaling modules of photoreceptor–transcription factors regulate light responses via different mechanisms in specific developmental contexts (Adopted from Jing and Lin, 2020)

Image caption: (a, d, f, g) Inhibition: the photoreceptors interact with transcription factors to inhibit their transcriptional activity. In (d), PIF4/5 activity is mildly inhibited, leading to hypocotyl elongation in canopy shade under low blue light. (b, c) Interference: light triggers interactions between photoreceptors and with AUX/IAA, interfering with the auxin-induced degradation of AUX/IAA by the E3 ligase SCFTIR, thereby repressing ARF activity and related auxin signaling. (e) Promotion: cry2 interacts with CIB1 and promotes the transcriptional activity of CIB1 in a blue light-dependent manner. For simplicity, phys and crys are depicted as monomers, although they are always present as dimers. Photoactivated monomeric UVR8 is transferred into the nucleus, leading to its association with light signaling components. Arrows and T-bars denote positive and negative regulation, respectively (Adopted from Jing and Lin, 2020)

4.2 Role of circadian rhythms in integrating environmental signals

Sometimes you will find that different rice varieties have different sensitivities to their internal clocks. But in any case, this circadian rhythm is like a time manager, aligning physiological activities with the changes of day and night and seasons. For example, it will refer to light and temperature signals together to adjust the rhythm of gene expression so that the rice plants are in the best growth state as much as possible (Venkat and Muneer, 2022). Speaking of key roles, we have to mention the "giant gene" (GI), which actually regulates PIF in the background, and PIF is the core machine for integrating light and temperature signals (Nohales et al., 2019). What's more interesting is that the circadian rhythm can also influence the "deployment" of chromatin in the cell nucleus, thereby determining which genes should be activated and when (Zhang et al., 2023). In the final analysis, this seemingly complex feedback loop and regulatory network allows rice to "predict" the sunrise and sunset every day in advance and to flexibly respond to seasonal changes (Creux and Harmer, 2019).

4.3 Epigenetic regulation in temperature-light adaptation

Sometimes, you will hear that the "switch" in rice depends not only on genes, but also on epigenetic "lubricants" to regulate the effects of light and temperature - don't think they are always rigid. Studies have found that histone modifications (such as H3K9 acetylation) do not simply remain unchanged, but fluctuate with the circadian rhythm. This oscillation happens to be consistent with the rhythm of gene expression, as if the two are "cooperating" to integrate environmental signals (Zhang et al., 2023). But don't forget that not all transcription factors are online on time every day-those transcription factors regulated by circadian rhythms have to have a dynamic party with chromatin to truly make epigenetic mechanisms come in handy in responding to climate change. What's more interesting is that core clock genes occasionally "link" with some transcription factors related to light and temperature signals, forming a seemingly messy but precise transcriptional regulatory network, highlighting the complex interaction between epigenetic regulation and environmental signal integration.

5 Case Study: Molecular Basis of Temperature and Light Adaptation in the Tropical Japonica Rice Cultivar

5.1 Selection criteria for the case study

Speaking of this case, it is tropical japonica rice that was chosen-the reason is simple. This kind of rice has its own unique "coping strategy" in the face of changes in temperature and light in tropical regions, and this is the key to its good survival and stable yield. Although it has undergone many genetic improvements, it has been able to gain a foothold under high temperature and unstable light conditions. It is these characteristics that make it an excellent "experimental subject" for studying the molecular mechanisms behind temperature and light adaptation. In fact, tropical japonica rice shows a lot of molecular and physiological adjustments that help it resist cold and heat stimulation. It is particularly worth mentioning that the activity of some specific genes and proteins makes it resistant to cold and heat, and its sensitivity to photoperiod has been improved, which further affects the yield.

5.2 Analysis of key findings

In tropical japonica rice, heat shock proteins (HSPs) play an indispensable role. They are actually like "repairmen" in cells, helping to stabilize those easily deformed proteins under high temperature stress, and even helping them to refold and return to normal. Don't underestimate this group of proteins. When the temperature soars, their number will increase rapidly, providing a protective umbrella for plants, helping them survive heat stress and ensuring that the basic functions of cells are not destroyed (Li et al., 2021; Sales et al., 2021).

Sometimes you may feel that tropical japonica rice is like a "time adjustment master" - it changes its sensitivity to the photoperiod, so that it can head and bear fruit on time even under conditions of flickering light. Let's talk about an exception first: not all tropical japonica rice relies on changing Hd1 to adjust, some varieties have other secrets. But the mainstream approach is to make the Heading date 1 (Hd1) gene inoperable. This deletion allele is not very popular in temperate japonica rice, but it comes in handy in the tropics. Under short-day conditions, this "broken" Hd1 prevents rice from flowering early, helping growers move the flowering time to a more appropriate time (Figure 2) (Kim et al., 2018). By the way, some people have specifically picked out the "magic" alleles of bZIP73 and COLD1 to deal with occasional cold air masses. This trick makes tropical japonica rice more frost-resistant and more productive (Sales et al., 2017; Liu et al., 2018).

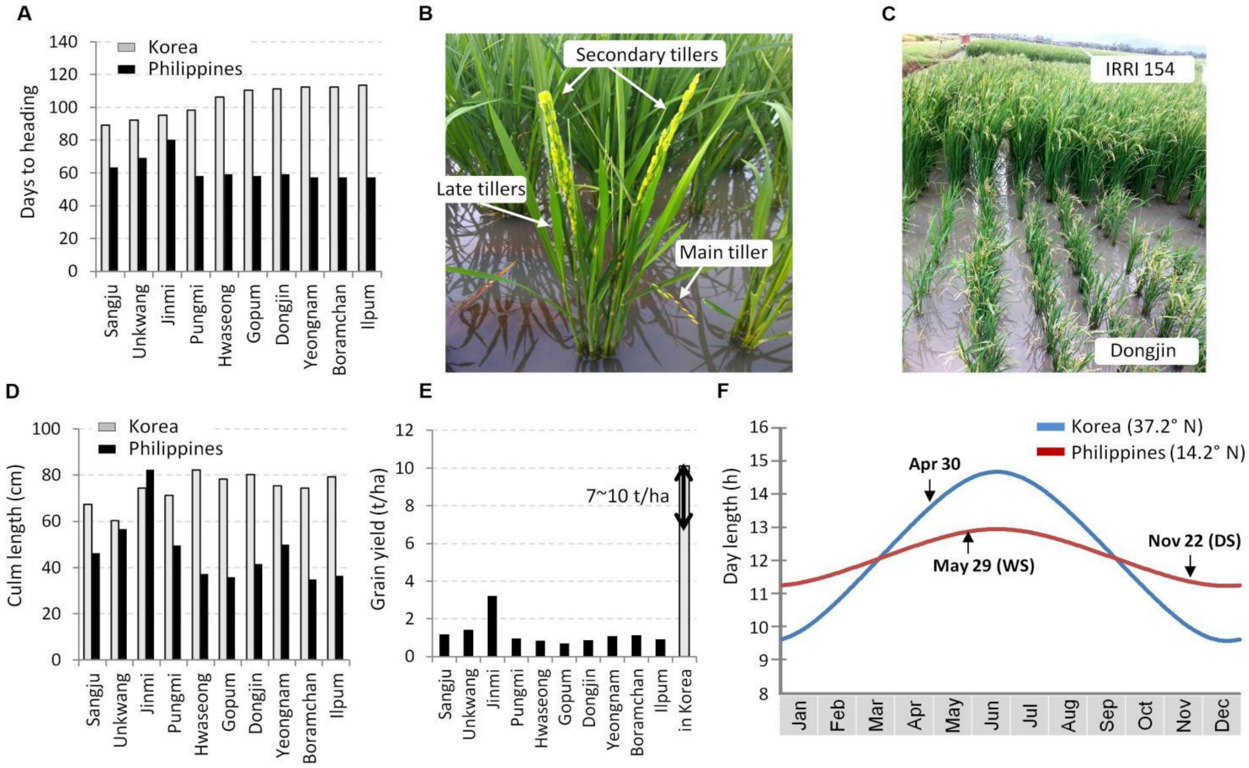

Figure 2 Phenotypes of the temperate japonica varieties in the tropical region (Adopted from Kim et al., 2018)

Image caption: Ten Korean temperate japonica varieties were tested in the dry season in Los Baños (14.2°N), Philippines and in Suwon (37.2°N), Korea. (A) Comparison of days to heading (DTH). (B) Irregular flowering among tillers (Dongjin variety). (C) Photo of the maturation stage. (D) Comparison of culm length. (E) Comparison of grain yield. Yield data in Korea were obtained from the rice variety information released by the RDA. (F) Seasonal day-length (h) of the RDA Suwon station and the IRRI headquarter in Los Baños. The dates of seeding are depicted in the graph. DS, dry season; WS, wet season (Adopted from Kim et al., 2018)

5.3 Implications for breeding programs and future research

When it comes to rice breeding, the tropical japonica rice variety is particularly interesting. Its special abilities to cope with high temperatures and strong light (molecular and physiological adaptability) are extremely useful for current breeding programs (Mao et al., 2019). However, to really make good use of these characteristics, we must first understand the genetic mechanisms behind them. Breeding experts are now doing two main things: on the one hand, they need to find favorable genetic variants (alleles) and find ways to get them into new varieties; on the other hand, they have to consider the background of climate change so that new varieties can adapt to more complex environments. Of course, traditional breeding alone may not be fast enough, so future research should focus on the following points: first, we need to find more genes and pathways involved in temperature and light adaptation, and second, we need to try new technologies such as gene editing, which may be able to breed more resistant varieties faster.

6 Adaptation Strategies for Enhanced Resilience

6.1 Breeding rice varieties for improved thermal and light tolerance

Climate change has brought considerable challenges to rice cultivation, especially high temperatures and abnormal light. Interestingly, however, researchers have found that some rice varieties are naturally very resistant - such as those that bloom in the morning, have large anthers and high pollen vitality (Khan et al., 2019). These characteristics are not just for show, they can really help rice cope with hot weather better. Now breeders are mainly starting from two directions: on the one hand, searching for germplasm resources with these excellent characteristics around the world, which is the most basic and important step; on the other hand, new technologies such as marker-assisted selection (MAS) and genetic modification are also gaining more and more attention (Zafar et al., 2020). After all, traditional breeding methods are slow to take effect, while molecular breeding can more accurately "install" the target traits into new varieties, greatly shortening the breeding cycle. In the final analysis, to cultivate rice that can truly adapt to the future climate, it may still rely on a combination of traditional methods and new technologies.

6.2 Gene editing approaches to enhance adaptive traits

When it comes to new weapons in rice breeding, CRISPR/Cas9 gene editing technology is definitely a tough guy. The most powerful thing about this technology is that it can accurately "prune" genes related to stress resistance (Rasheed et al., 2021; Nascimento et al., 2023). For example, a recent study combined resistance genes such as PgHSF4 and p68, and the resulting rice was not only resistant to high temperatures, but also to salinity and oxidative stress (Sheela et al., 2023). However, gene editing alone is not enough, and you have to know which genes to edit first. At this time, QTL positioning comes in handy-by analyzing a large amount of data to find key quantitative trait loci (Raza et al., 2020; Liu et al., 2023). With these target genes, the efficiency of breeding heat-resistant rice can be greatly improved by using CRISPR technology for precise modification. Interestingly, although these new technologies look very "high-end", they still need to be combined with traditional breeding experience in actual operation. After all, rice's stress resistance is a complex trait that cannot be completely solved by simply changing one or two genes.

6.3 Agronomic practices to mitigate the impact of temperature and light stress

Faced with increasingly frequent high temperatures and abnormal light, farmers actually have many practical coping strategies. Let's start with the simplest one - adjust the sowing time to avoid the hottest days, and then choose some varieties that are used to blooming in the morning (Khan et al., 2019), so that the rice can suffer less. However, these are not enough. Now agricultural technicians will also use some "little tricks", such as spraying specific plant hormones, or adding some special fertilizers and protective agents (Rasheed et al., 2021), which can help rice better resist the harsh environment. Interestingly, more sophisticated control methods have emerged in recent years. Through specific growth regulators and agronomic operations (Zafar et al., 2020), rice can be "trained" to adapt to environmental changes. Of course, if these traditional methods can be used in combination with new technologies such as gene editing, the effect will be better. After all, to cope with the big challenge of climate change, we still have to take a multi-pronged approach.

7 Knowledge Gaps and Future Research Directions

7.1 Unresolved questions in temperature and light sensing mechanisms

Although a lot of research has been done on the response mechanism of rice to changes in light and temperature, there are still many mysteries to be solved. Take cold tolerance as an example. Scientists have discovered a strange phenomenon - light signals and biological clocks obviously affect cold tolerance, but how they specifically regulate related genes is still an unsolved mystery (Lu et al., 2020). Even more troublesome is the study of heat tolerance of reproductive tissues. Although some key sites have been found through QTL positioning (Raza et al., 2020), how these genes work specifically still needs further exploration. When it comes to plant hormones, it is even more complicated. Hormones such as auxin and ABA behave quite "changeable" under high temperatures (Sharma et al., 2021). The regulatory network they form is like a black box. We only see the input and output, and the process in between is still unclear. Filling these research gaps may help us breed more stress-resistant rice varieties.

7.2. Potential of integrating omics approaches

To truly understand how rice copes with changes in temperature and light, a single research method is not enough. Genomics has indeed helped us find a number of QTL loci related to stress resistance (Lv et al., 2019; Raza et al., 2020), but knowing these alone is not enough. For example, transcriptome sequencing allows us to see which genes are "working overtime" under high temperatures, but what effects these genes actually produce still needs to be verified by proteomics (Sharma et al., 2021). Interestingly, when these omics data are analyzed together, unexpected discoveries are often made. The complex "interactions" between different stress resistance pathways cannot be discovered by looking at a single set of data (Ullah et al., 2022; Usman et al., 2023). Just like a jigsaw puzzle, only when all the pieces are put together can the complete picture be seen. This multi-omics approach may really help us breed tougher rice varieties.

7.3 Importance of field studies in understanding real-world adaptation

Although laboratory research can control variables, if we really want to understand the stress resistance of rice, we still have to look at its performance in the field. After all, nature is not as "rules-based" as the laboratory - the temperature fluctuates, the light is sometimes strong and sometimes weak, and the humidity changes at any time (Hashida et al., 2021). Interestingly, different rice varieties can react very differently to rising CO2 and temperature, which shows that the "compatibility" of genes and the environment is really critical. What's even more surprising is that some varieties that perform mediocrely in the laboratory are very resistant in the field. These phenomena can only be discovered through long-term field observations (Guo et al., 2020), because the gene interaction network in the field is much more complex than that in the laboratory. Therefore, in breeding research now, it is best to combine laboratory data, omics analysis and field verification, so that the varieties cultivated are more likely to adapt to real climate change.

8 Conclusion

Sometimes it's incredible to think about it. Rice supports the livelihoods of more than half of the population, but it also has to face the dual challenges of light and temperature at the same time. Don't think that rice fields in every place are the same. Some varieties are not very sensitive to cold. In short, light signals and the "clock" in the body need to work together to allow rice plants to survive cold stress, but the details of this "tacit cooperation" need to be further analyzed. Look at the high temperature again. It is easy to go wrong when it comes to heading and flowering, so we have to work hard at the genetic and molecular levels to cultivate new varieties that can withstand the heat. Transcriptome data show that auxin and ABA response genes play a key role in heat stress, and also provide clues to unlock the secret key of heat resistance genes. Finally, don't forget that genetic improvement alone is not enough: in the context of climate change, optimizing agronomic management is also a necessary means to improve the yield and quality of japonica rice.

Don't think that rice can automatically adapt to all environments - its ability to "resist stress" in temperature and light is actually closely related to our rice bowl. Some people say that as climate change makes cold and heat more extreme, this matter is more urgent; but don't forget that the situation in different places is not exactly the same. When high temperatures come, heading and filling are easily affected; if it encounters cold and crisp, the yield will be discounted, which is particularly fatal for those areas that rely on rice for food. Since relying solely on natural regulation is thankless, scientists have taken action: through genetic and molecular strategies, new varieties that are more heat-resistant and cold-resistant are cultivated to stabilize the harvest. At the same time, there are teams that are deeply analyzing the genes and regulatory networks related to temperature stress responses, striving to point out a clear way for breeding. The ultimate goal is simple - to make rice more able to withstand future extreme weather and stop stretching food security.

Sometimes you wonder, where should the focus of future rice research be placed? Don't rush to focus only on the "molecular network", first think about how it actually carries together the three mountains of light signals, the "clock" in the body and temperature stress. Although genomics, transcriptomics and proteomics continue to give us new weapons, don't ignore the "lessons learned" from model plants (such as Arabidopsis) - of course, directly copying them won't work. More importantly, laboratory data alone is not enough, and researchers, breeders and policymakers must be brought to a table to turn those "discoveries in the laboratory" into solutions that can be used in the field. Only in this way can we truly enhance the resistance of rice to stress and ensure that the global food supply will not fall behind in the future of climate change.

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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.

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