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Transcriptional Regulation and Gene Networks in Rice under Water Deficit Conditions

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Abstract Water scarcity is a major limiting factor in agriculture, significantly affecting crop productivity and food security. As a major food crop, rice is particularly sensitive to water stress, so it is of great significance to understand its transcriptional regulation and gene network under such conditions. This review aims to systemic synthesis the transcriptional response and gene network of rice under water deficit conditions to reveal its drought resistance mechanism. We summerized the studies on functional analysis of key drought resistance genes and validation of candidate genes using CRISPR-Cas9 technology. Additionally, the roles of abscisic acid (ABA) and other hormones in gene regulation has also been explored. Despite the inherent challenges of omics data analysis, advances in genomics and transcriptomics technology support recent research. The findings have significant practical implications for cultivating drought-resistant rice, and provide potential biotechnological applications. Future research should focus on emerging technologies and innovative strategies to further improve the resilience of crops to water scarcity.

Keywords Rice; Water deficit; Transcriptional regulation; Gene networks; Drought tolerance

1 Introduction

In the context of increasingly severe global climate change, water deficit has become one of the significant abiotic factors hindering the sustainable development of agriculture. Water deficit, commonly referred to as drought stress, affects various physiological and biochemical processes in plants, leading to reduced growth, development, and yield. Rice, one of the world's most important food crops, is severely affected by water deficit, resulting in a significant decline in grain yield and quality, posing a threat to food security (Hadiarto and Tran, 2011; Gaballah et al., 2020; Sakran et al., 2022). Therefore, developing drought-tolerant rice varieties is crucial for maintaining rice production under water-limited conditions and ensuring global food security (Gaballah et al., 2020; Sakran et al., 2022).

Rice, with its remarkable high-yield potential and excellent grain quality, is widely favored by farmers and consumers, becoming an indispensable precious crop in the agricultural field. However, its high sensitivity to water deficit requires us to delve deeply into its genetic and physiological responses under drought stress to better address the challenges posed by drought. Research on the transcriptional regulation and gene networks of rice under water deficit can provide a deeper understanding of drought resistance mechanisms and offer strong theoretical support for cultivating rice varieties with enhanced drought resistance (Wang et al., 2011; Chen et al., 2021). For instance, some studies have revealed key genes and regulatory pathways in rice under drought conditions, providing new insights for rice drought resistance breeding (Silva et al., 2022). In addition, researchers have comprehensively analyzed the molecular responses of rice to drought using genomics, transcriptomics, and metabolomics technologies. By comparing the gene expression differences between drought-resistant and sensitive rice varieties under drought stress, a series of genes related to drought resistance have been identified. For example, some studies have focused on key genes in the ABA signaling pathway, as ABA plays a crucial role in plant responses to drought stress (Xu et al., 2019).

This study is expected to explore the transcriptional regulation and gene network of rice under water deficient conditions, identify key transcription factors (TFs) and genes differentially expressed under drought stress, and comprehensively analyze the genetic diversity and combinatorial ability of different rice genotypes under water deficit conditions, in order to accelerate the breeding of drought-resistant rice varieties to improve rice yield and ensure food security under climate change conditions.

2 Water Deficit Stress in Plants

2.1 Definition and types of water deficit

Water deficit, that adversely affects plant growth and productivity. It occurs when the water availability in the soil is insufficient to meet the plant’s needs, they begin to experience drought stress, leading to a range of physiological and biochemical changes. Water deficit can be classified into different types based on its duration and intensity, including transient, moderate, and severe drought conditions. Transient drought， where plants face short-term water shortage, can often be overcome by their own physiological regulatory mechanisms, such as adjusting the opening and closing of stomata to reduce water loss. Moderate drought, with a continuous decrease in soil moisture, results in a significant slowdown in plant growth rate, possible wilting of leaves, reduced photosynthetic efficiency, and limited root expansion, requiring human intervention through irrigation to ensure normal plant growth. Severe drought, where the soil loses almost all its moisture, leaves plants unable to obtain sufficient water through natural means. This not only leads to stagnate in plant growth but may also cause widespread plant death, having a profound impact on agricultural production and ecosystems. The latter two types of drought have a serious impact on plant development and yield (Volante et al., 2017; Sakran et al., 2022).

2.2 Physiological and biochemical impacts of water deficit on plants

Water deficit stress triggers a complex array of physiological and biochemical responses in plants. Physiologically, it leads to reduced leaf water potential, stomatal closure, decreased photosynthetic rate, and impaired growth (Basu et al., 2016; Zhu et al., 2021). Biochemically, plants under water deficit conditions exhibit increased synthesis of ABA, which plays a crucial role in mediating stress responses. ABA induces the expression of genes involved in osmotic adjustment, cellular dehydration tolerance, and protective enzyme production (Baldoni et al., 2016; Seeve et al., 2017). Additionally, water deficit stress results in the accumulation of reactive oxygen species (ROS), which can cause oxidative damage to cellular components. Plants maintain normal physiological functions by enhancing the activity of antioxidant enzymes, synthesizing other antioxidants (such as ascorbic acid and glutathione), producing osmoprotectants (such as proline and soluble sugars), and synthesizing specific osmoregulatory substances (such as betaines and polyols) (Mittler, 2002; Yun et al., 2010; Kim et al., 2019; Basu and Roychoudhury, 2021).

2.3 Specific challenges and responses in rice

Rice, as a crop with high water demand, is highly sensitive to water deficit stress, which poses severe challenges to its growth and productivity. Under water deficit conditions, rice exhibits significant reductions in traits such as plant height, chlorophyll content, relative water content, and grain yield. Conversely, traits like leaf rolling and sterility percentage increase, indicating the negative impact of water deficit environment on rice growth (Volante et al., 2017; Hoang et al., 2019). The genetic response to water deficit in rice involves the regulation of numerous genes and TFs that control stress-responsive pathways. For instance, genes related to ABA biosynthesis, osmotic adjustment, and ROS detoxification are upregulated to enhance drought tolerance. Moreover, genome-wide association studies (GWAS) have identified specific quantitative trait loci (QTLs) associated with drought tolerance traits, which can be targeted in breeding programs to develop rice varieties with improved drought tolerance (Volante et al., 2017; Hoang et al., 2019). Through hybridization and backcrossing, some drought-tolerant traits have been transferred to modern rice varieties, improving their performance under drought conditions. By using CRISPR/Cas9 gene-editing technology, genes that inhibit rice drought tolerance have been knocked out, thereby enhancing their growth and yield under drought conditions. The combination of physiological, biochemical, and genetic approaches is crucial for improving rice drought tolerance and ensuring stable yields under water deficit conditions.

3 Transcriptional Regulation under Water Deficit

3.1 Overview of transcriptional regulation in plants

Plant transcriptional regulation involves TFs including MYB, bZIP, AP2/ERF, NAC, etc., controlling gene expression. These TFs bind to specific DNA sequences near the genes they regulate, known as cis-regulatory elements, such as promoter regions, thereby regulating the expression of downstream genes. This process is crucial for plants to adapt to various environmental stresses, including water deficit. TFs can activate or repress the transcription of target genes, leading to changes in the levels of mRNA and, consequently, protein production. These changes enable plants to modify their physiological and developmental processes to cope with stress conditions (Yun et al., 2010; Wilkins et al., 2016; Favreau et al., 2023). In addition to TFs themselves, there are complex signaling pathways in plants that sense environmental changes and transmit signals to TFs, thereby initiating or inhibiting the expression of specific genes (Cutler et al., 2010). Moreover, epigenetic regulatory mechanisms also play a vital role in plants' response to environmental stresses. Epigenetic changes such as DNA methylation and histone modification can affect chromatin structure, thus influencing the binding ability of TFs to DNA and regulating gene expression (Kim et al., 2015).

3.2 Key TFs involved in water deficit response

Several key TFs have been identified as crucial players in the response of plants to water deficit. For instance, the NAC TF ONAC022 has been shown to improve drought tolerance in rice by modulating an ABA-mediated pathway, leading to increased proline and soluble sugar contents, reduced water loss, and enhanced survival under drought conditions (Hong et al., 2016). Additionally, MYB TFs are involved in regulating drought-inducible genes, contributing to the plant's ability to withstand water deficit (Smita et al., 2015). Dehydration-responsive element-binding protein (DREB) can bind to specific DNA sequences under drought conditions, thereby initiating the expression of a series of defense genes to enhance plant drought tolerance (Lata and Prasad, 2011). WRKY TFs participate in various physiological processes of plants, including responses to drought, by regulating the expression of downstream target genes. For example, *WRKY46* in *Arabidopsis* has been found to enhance plant drought tolerance by activating the expression of antioxidant enzyme genes (Liu et al., 2019). TFs of the AP2/ERF family play a significant role in plant responses to drought stress. They can recognize and bind to drought response elements (DRE) in gene promoters, thus activating the expression of a series of drought-related genes (Yoshida et al., 2014). Other important TFs include bZIP, ERF, etc., which can regulate various stress response genes and pathways (Seeve et al., 2017; Chen et al., 2021).

3.3 Regulatory networks and pathways activated under water deficit

The regulatory networks activated under water deficit conditions are complex and involve multiple layers of regulation. Environmental gene regulatory influence networks (EGRINs) have been identified in rice, which coordinate the timing and rate of gene expression in response to water deficit. These networks include interactions between many TFs and their target genes, integrating signals from various stress-responsive pathways (Wilkins et al., 2016). For example, the TCONS_00021861/miR528-3p/YUCCA7 regulatory axis has been identified as a key component of the drought response in rice, where the lncRNA TCONS_00021861 acts as a competing endogenous RNA to regulate the expression of YUCCA7 (Figure 1), leading to increased auxin (IAA) production and enhanced drought tolerance (Chen et al., 2021).

The figure in Chen et al. (2021) shows the effects of overexpression TCONS_00021861 and miR528-3p on rice seedling growth indexes and ROS accumulation under drought stress. The dry weight, root length, leaf length, and H2O2 and O2 contents of roots and leaves were observed in different treatment groups by simulating drought stress by PEG. The results showed that overexpression of TCONS_00021861 and miR528-3p significantly affected the growth and ROS accumulation of rice seedlings under drought conditions. Specifically, the LNC and MIR treatment groups showed different response patterns in root and leaf growth, while the LNC+MIR combined treatment further enhanced the drought response. These results suggest that TCONS_00021861 and miR528-3p may affect drought adaptation of rice by regulating ROS accumulation and signaling pathways, revealing important signaling pathways involved in the response to water shortage.

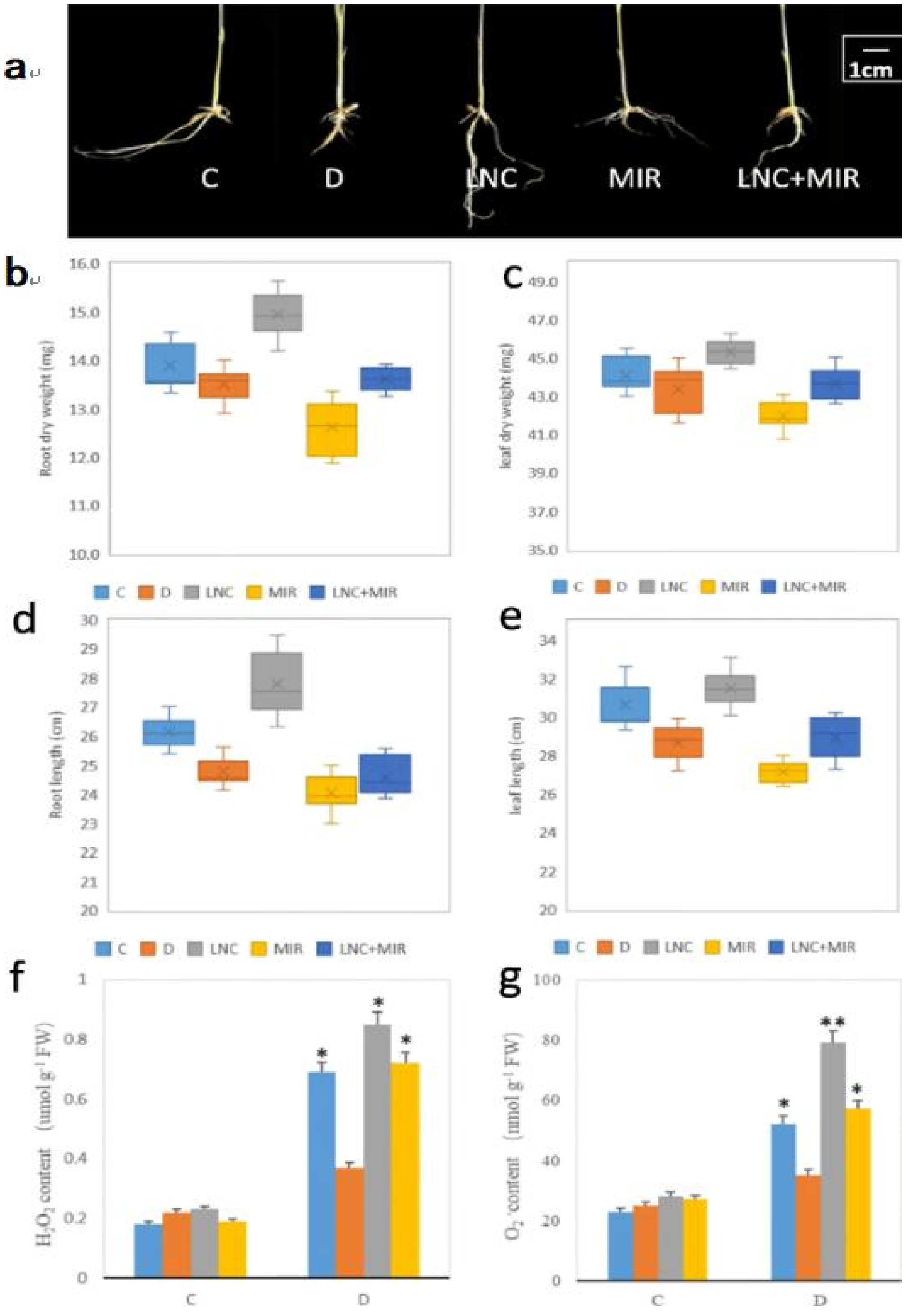

Figure 1 Effects of overexpressed TCONS_00021861 and miR528-3p on growth indices and ROS accumulation of drought-stressed rice seedlings (Adopted from Chen et al., 2021)

Image caption: a: Seedlings were photographed and measured 1 week after PEG simulated drought stress. b: root dry weight. c: leaf dry weight. d: root length. e: leaf length. f: H_2O_2 content. g: O^{2-} content. (Adopted from Chen et al., 2021)

While, the TCONS_00029371/miR408-5p/LOX2 regulatory axis has been revealed as a core element in rice drought resistance strategies. In this regulatory axis, the long non-coding RNA TCONS_00029371 functions as a competitative endogenous RNA (ceRNA), fine-tuning the expression level of LOX2, thereby regulating lipid peroxidation and antioxidant responses to enhance rice drought tolerance. This discovery provides a new perspective on how rice adapts to drought conditions (Li et al., 2022). The integration of transcriptomic, metabolomic, and physiological data has revealed genotype-specific regulatory mechanisms affecting rice development and response to water deficit, highlighting the importance of cell wall metabolism and other fundamental processes (Favreau et al., 2023; Wang et al., 2023).

4 Gene Expression Dynamics in Rice

4.1 Differential gene expression under water deficit conditions

Rice exhibits significant changes in gene expression when subjected to water deficit conditions. A study using genome-wide gene expression profiling identified 5 284 genes that were differentially expressed under drought stress, with many of these genes being tissue- or stage-specific (Basu and Roychoudhury, 2021). The latest whole-genome sequencing analysis has identified 6 127 genes that change specifically under drought stress, including not only universally responding “star” genes but also many previously overlooked genes with potentially important functions (Chen et al., 2023). Another research highlighted the differential expression of genes in hybrid rice LYP9, where 595 genes were up-regulated and 25 down-regulated under stress conditions, indicating a complex regulatory network that enhances stress tolerance (Sakran et al., 2022). Additionally, a GWAS identified specific marker-trait associations (MTAs) related to drought tolerance, further elucidating the genetic basis of stress response in rice (Volante et al., 2017). Specifically, a study pointed out that in the super rice variety “Jin You”, 783 genes were significantly upregulated and 117 genes were significantly downregulated under drought conditions, revealing the differences and complexity of drought tolerance mechanisms among varieties (Li et al., 2022).

4.2 Temporal and spatial patterns of gene expression

The temporal and spatial patterns of gene expression in rice under water deficit conditions are highly dynamic. For instance, the expression of drought-responsive genes varies significantly across different tissues and developmental stages. In one study, the transcriptome from leaf, root, and young panicle at three developmental stages was analyzed, revealing that most differentially expressed genes (DEGs) were regulated in a tissue- or stage-specific manner. They found that under drought conditions, the expression of *OsNAC6* gene in leaves was significantly upregulated, which is a known transcription factor that positively regulates drought response, and its upregulation may have enhanced the drought tolerance of leaves. In roots, the expression of *OsDREB2A* gene was more significant, which is closely related to root development and water absorption, and its upregulation may have promoted root growth and water absorption capacity under drought conditions (Yin et al., 2021). Another study on maize, which can be extrapolated to rice, showed that TFs were differentially regulated across stressed seedling tissues, indicating a complex temporal and spatial regulation of gene expression. They observed that under drought stress, the expression of *ZmDREB2* gene in corn seedling leaves was upregulated, while its expression in roots remained relatively stable. This differential regulation may reflect different strategies and priorities of different tissues in coping with drought stress (Seeve et al., 2017).

4.3 Methods for studying gene expression

Several advanced methods are used to study gene expression in rice under water-scarce conditions: RNA-seq, a high-throughput sequencing technology, allows for a comprehensive analysis of the transcriptome and the identification of known and new transcripts. It has been widely used to analyze gene expression conditions in rice under various stresses (Wilkins et al., 2016). Quantitative PCR (qPCR) is used to quantify the expression level of a specific gene. It is particularly useful for validating RNA-seq results and studying the expression of key regulatory genes under stress. Gene expression sequence analysis (SAGE), a technique used to study the transcriptome of hybrid rice LYP9 and its parent varieties, provides insight into the differential expression of genes (Marcon et al., 2016). Whereas, network component analysis (NCA) integrates measurements at multiple genome scales to infer EGRINs that coordinate gene expression in response to environmental signals.

5 Regulatory Networks in Response to Water Deficit

5.1 Gene-gene interaction networks

Gene-gene interaction networks play a crucial role in the response of rice to water deficit conditions. EGRINs have been identified as key players in coordinating the timing and rate of gene expression in response to environmental signals, including water deficit. EGRINs integrate multiple layers of regulation, resulting in changes in transcript levels. For instance, in tropical Asian rice cultivars, EGRINs were inferred by integrating time-series transcriptome data, patterns of nucleosome-free chromatin, and known cis-regulatory elements, identifying 5447 putative target genes for 445 TFs (Wilkins et al., 2016; Ueda et al., 2020). Additionally, MYB

TFs have been shown to regulate defense responses to various stresses, including drought, by forming complex co-regulatory networks with other genes. For instance, the expression of *OsMYB2* is significantly upregulated under drought conditions, and this upregulation promotes the expression of multiple genes related to drought tolerance. Reducing the expression level of *OsMYB2* using RNAi technology significantly decreased the drought tolerance of rice plants, demonstrating the key role of *OsMYB2* in drought response (Yoo et al., 2017).

5.2 Signaling pathways involved in water deficit response

Several signaling pathways are activated in response to water deficit in rice. One notable pathway involves the OsPhyB-mediated regulatory mechanism, which controls drought tolerance in rice roots. This pathway was identified through RNA-Seq analysis, revealing that OsPhyB represses the activity of ascorbate peroxidase and catalase, which are crucial for ROS processing and drought tolerance (Yoo et al., 2017). Another significant pathway involves the oxidative-mediated network, which configures early responses to stress. This network includes bZIP, ERF, and MYB TFs that respond to oxidative signals, playing a critical role in the early stages of stress response (Basu and Roychoudhury, 2021).

In the complex regulatory network of rice's response to drought, in addition to the aforementioned signaling pathways, there is also a pathway involving ABA signaling. ABA is a plant hormone that plays a central role in plants' response to water stress. Studies have shown that ABA, by activating specific receptor proteins, initiates downstream signaling cascades to regulate the expression of a series of genes involved in processes such as water retention, stomatal closure, and root growth. For example, ABA response element-binding proteins (AREB) and positive regulatory factors (ABF) TFs play a key role in the ABA signaling pathway, activating the expression of multiple drought-related genes (Manuel et al., 2023). Additionally, calcium signaling pathways also play an important role in rice's response to drought. Under drought conditions, changes in intracellular calcium ion concentrations can act as second messengers to activate calcium-dependent protein kinases (CDPKs) and calmodulins (CaMs). These calcium signaling molecules further regulate downstream TFs and effector proteins, thus affecting the plant's physiological responses.

For instance, OsCPK17 and OsCaM1-1 have been shown to play a positive role in rice drought resistance (Asano et al., 2012). Besides the aforementioned signaling pathways, plant hormones gibberellin (GA) and IAA also play a role in regulating rice's response to drought. Under drought conditions, the synthesis and metabolism of GA are inhibited, leading to slowed plant growth and reduced water consumption. Meanwhile, the redistribution of IAA and changes in its signaling pathways help regulate root growth and development to adapt to the water-stressed environment (Song et al., 2024). The integration of these signaling pathways helps regulate rice growth and maintain vitality under water-deficient conditions.

5.3 Integration of omics data to construct regulatory networks

The construction of regulatory networks in response to water deficit in rice has been significantly advanced by the integration of various omics data. For example, the integration of transcriptome data, nucleosome-free chromatin patterns, and cis-regulatory elements has been used to infer EGRINs, which include regulatory interactions between thousands of target genes and hundreds of TFs (Wilkins et al., 2016). Additionally, the use of high-throughput expression profiling data has facilitated the construction of co-expression networks, revealing the regulatory hierarchies of MYB TFs under stress conditions. The integration of omics data, such as RNA-seq and promoter-GUS reporter systems, has also been employed to validate the expression patterns of candidate genes and monitor stress responses, further enhancing our understanding of the regulatory networks involved in drought tolerance (Chen et al., 2021).

6 Case Studies of Key Genes and Pathways

6.1 Functional analysis of key drought-responsive genes

In the context of rice under water deficit conditions, several key drought-responsive genes have been identified and functionally analyzed. For instance, the study by identified 5447 putative target genes for 445 TFs involved in the response to water deficit (Volante et al., 2017). These genes are regulated through EGRINs, which coordinate gene expression in response to environmental signals (Figure 2) (Favreau et al., 2023).

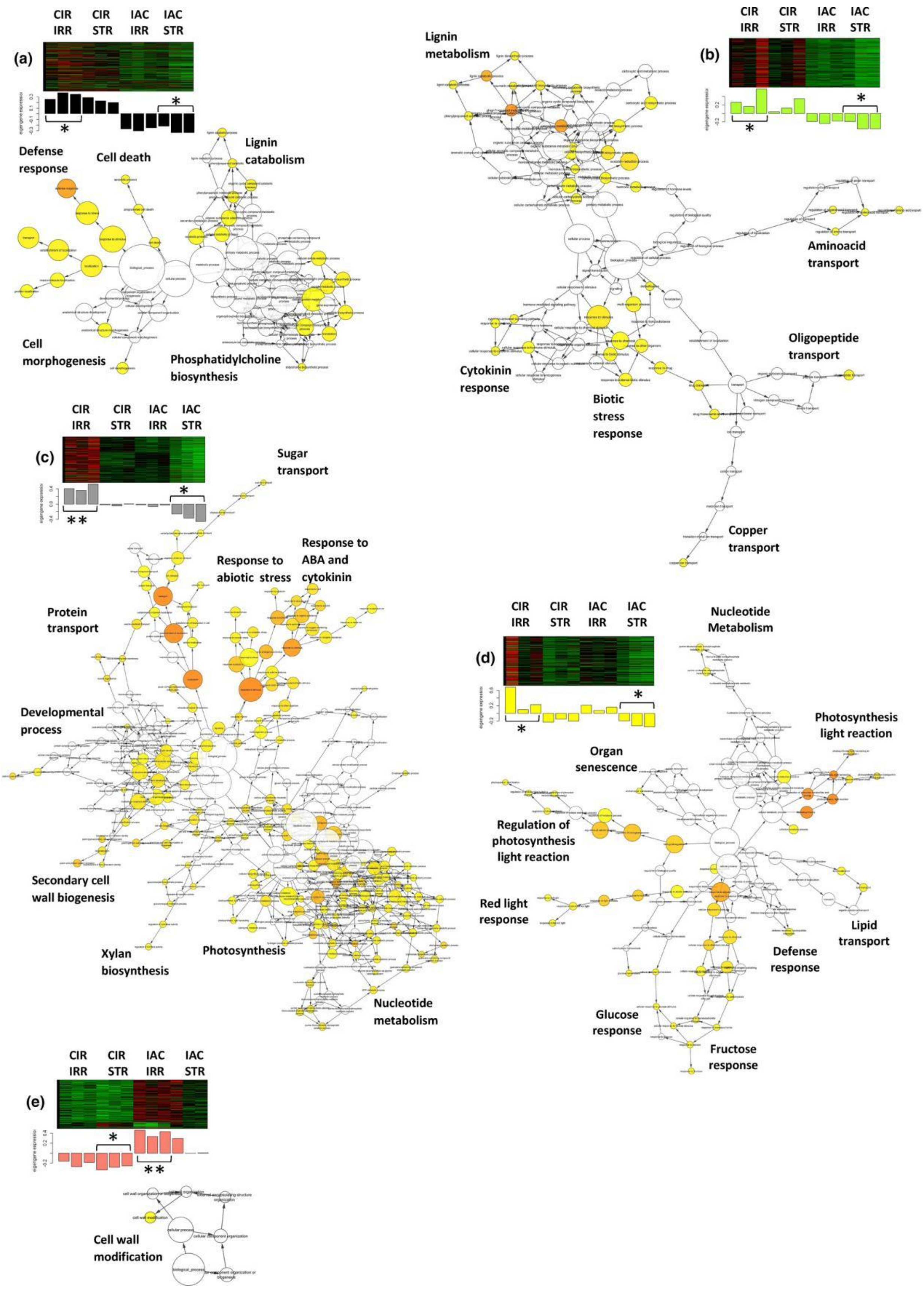

Figure 2 The gene ontology enrichment of the network (Adopted from Favreau et al., 2023)

Image caption: Mean genes in CIR-IRR are overregulated: (a) black (*p*-value=2.1e-2); (b) yellow-green (*p*-value=1.6e-2); (c) Grey 60 (*p*-value=3.4e-4); (d) yellow (*p*-value=1.6e-2); and IAC-IRR: (e) salmon (*p*-value=1.2e-3). (*p*-value: ns>0.05; *<0.05; **<0.01; ***<0.001) (Adopted from Favreau et al., 2023)

Additionally, highlighted the role of various TFs, including members of the NAC family, which are upregulated during water-deficit stress and are key regulators of multiple abiotic stresses. The study further identified 5 284 DEGs under drought stress, with many being tissue- or stage-specific, indicating a complex regulatory network that responds to water deficit conditions. *DRG9* is a newly discovered drought-resistant gene that encodes a double-stranded RNA-binding protein. Under drought stress, *DRG9* aggregates into stress granules through liquid-liquid phase separation, positively regulating the drought resistance of rice. The *DRG9-DR* allele is enriched in upland rice, and its introduction into cultivated varieties can significantly improve drought resistance (Wang al., 2024). Additionally, genes such as *OsDREB1G*, *OsNAC6*, *OsLEA3*, *OsPP2C, DROT1,* and *qRT9* play key roles in rice's response to drought (Xu et al., 2016; Sun et al., 2022; Wang et al., 2024).

Favreau et al. (2023) demonstrated gene expression and gene ontology (GO) enrichment analysis in rice under different genotypes and water treatment conditions. Under CIR-IRR and IAC-IRR conditions, genes for multiple biological processes and molecular functions, such as lignin metabolism, cell death, defense response, photosynthesis, etc., were significantly overexpressed, which were important biological processes in response to drought stress. The color of each network node represents the corrected p-value, yellow to orange indicates significant enrichment, and white nodes represent the parent node of the significant enrichment category. The histogram shows the mean gene expression for each sample and the significance levels for different genotypes and water treatments. Under drought conditions, rice adapts to a water-deficient environment by regulating several key genes. These genes are involved in defense response, hormone signaling, photosynthesis, and modification of cell structures, providing important information for understanding the mechanisms of drought response.

6.2 CRISPR-Cas9 mediated validation of candidate genes

CRISPR-Cas9 technology has been employed to validate candidate genes involved in drought tolerance. The study identified several candidate genes such as *OsOFP2*, *Dlf1*, and *OsMADS56*, which were associated with limited water conditions. These genes were further validated using CRISPR-Cas9 to confirm their roles in drought tolerance. Similarly, Sakran et al. (2022) utilized genomic and transcriptomic approaches to identify and validate 36 DEGs with specific inherited alleles in the drought-resistant rice maintainer HuHan2B. These DEGs were involved in the regulatory network of TFs and target genes, demonstrating the potential of CRISPR-Cas9 in validating and improving drought tolerance traits. Another study focused on candidate genes such as *OsDREB2A*, *OsNAC6*, and *OsPP2C*. Using CRISPR-Cas9 technology, specific sgRNAs were designed to target specific sequences of these genes to validate their drought resistance functions. The knockout of the *OsDREB2A* gene led to significant changes in the gene expression profile of rice under drought conditions, especially the significant downregulation of gene expression related to drought response, indicating that *OsDREB2A* plays a central role in transcriptional regulation under drought stress (Cui et al., 2011). The knockout of the *OsNAC6* gene affected the root development and water absorption capacity of rice, weakening the plant's viability under drought conditions and further confirming the positive role of *OsNAC6* in drought resistance (Nakashima et al., 2007). In summary, CRISPR-Cas9 technology provides an efficient and precise method for validating candidate genes for drought resistance.

6.3 Role of ABA and other hormones in gene regulation

ABA and other hormones play crucial roles in the regulation of gene expression under drought conditions. The study identified TFs involved in both ABA-dependent and ABA-independent pathways that are upregulated during water-deficit stress (Ray et al., 2021). This includes members of the zinc-finger TFs and the NAC TF family, which are key regulators of abiotic stress responses. Additionally, Seeve et al. (2009) highlighted the involvement of ABA and auxin-dependent signaling pathways in regulating root growth responses to water deficits. Park et al. (2009) revealed the interaction between ABA receptors PYR/PYL/RCAR and PP2C protein phosphatases and SnRK2 protein kinases, which together regulate the physiological processes of stomatal closure and root growth, thereby enhancing the drought resistance of rice. Another study emphasized the differential expression of genes involved in hormone-mediated signaling and lignin biosynthesis, which is crucial for rice's tolerance response to osmotic stress (Baldoni et al., 2019). In addition to ABA and IAAs, GAs, ethylene (ETH), and cytokinins (CTK) also play important roles in plant responses to drought stress. For example, under drought conditions, the expression of certain genes in the GA signaling pathway is suppressed, thus slowing down plant growth to conserve energy and water resources. ETH production increases under drought conditions, and it regulates plant defense responses by activating a series of TFs, such as ERF family members. These TFs can initiate the expression of a series of downstream genes, thereby enhancing plant drought tolerance (Kumar et al., 2023). Furthermore, research also indicates that there are complex interactions and cross-regulatory networks among these hormones. For instance, ABA and GAs can exhibit antagonistic effects in certain situations, with GAs inhibiting ABA-induced stomatal closure, while ABA can inhibit GA-promoted cell elongation. This interaction between hormones is crucial for plant adaptation under different environmental conditions (Wang et al., 2018). Through these studies, we can not only better understand the role of plant hormones in gene regulation but also provide a theoretical basis for cultivating drought-resistant crop varieties.

7 Advances in Genomics and Transcriptomics Technologies

7.1 High-throughput sequencing technologies

High-throughput sequencing technologies have revolutionized the field of genomics and transcriptomics, enabling comprehensive analysis of genetic and transcriptomic data at an unprecedented scale. Next-generation sequencing (NGS) platforms have facilitated the generation of vast amounts of data, which are crucial for understanding the complex regulatory networks in rice under water deficit conditions. For instance, the integration of time-series transcriptome data with patterns of nucleosome-free chromatin and cis-regulatory elements has allowed the inference of EGRINs in rice, which coordinate gene expression in response to environmental stress (Ahn et al., 2017). Additionally, GWAS have identified significant MTAs in rice under different water management systems, providing insights into the genetic basis of traits related to water deficit adaptation (Wang et al., 2022). Utilizing RNA-seq technology in conjunction with epigenetic markers such as DNA methylation and histone modification data provides a more comprehensive understanding of the gene expression regulatory mechanisms in rice under water deficit conditions. High-throughput sequencing technology has also facilitated the application of gene-editing tools like CRISPR/Cas9 in rice breeding. By precisely modifying specific genes in the rice genome, scientists can develop rice varieties with enhanced drought resistance. In summary, high-throughput sequencing technology has become an indispensable tool in studying rice drought resistance.

7.2 Bioinformatics tools for data analysis

The explosion of multi-omics data necessitates advanced bioinformatics tools for effective data analysis and integration. Various computational methods and platforms have been developed to manage and analyze these large datasets. For example, bioinformatics pipelines have been employed to analyze multi-omics data in transgenic rice, revealing how specific TFs like *OsERF71* regulate global gene expression to confer drought resistance (McDaniel et al., 2021). Tools such as Anvio offer advanced analysis and visualization capabilities, enabling researchers to interactively organize and explore complex omics data (Eren et al., 2015). Moreover, the integration of multi-omics approaches, including genomics, transcriptomics, proteomics, and metabolomics, has been instrumental in elucidating the molecular mechanisms underlying stress responses in crops (Liu et al., 2020; Yang et al., 2021).

7.3 Challenges and future directions in omics research

Despite the significant advancements, several challenges remain in the field of omics research. One major challenge is the integration and interpretation of multi-omics data, which requires sophisticated computational tools and expertise. The rapid pace of technological development can be daunting for researchers new to the field, highlighting the need for user-friendly platforms and comprehensive training (McDaniel et al., 2021). Additionally, the complexity of regulatory networks and the dynamic nature of gene expression under stress conditions necessitate continuous improvement in data analysis methods. Future research should focus on developing more robust and integrative bioinformatics tools to enhance our understanding of gene networks and transcriptional regulation in crops. The integration of systems biology approaches with multi-omics data holds promise for predicting complex traits and improving crop resilience under environmental stresses (Yang et al., 2021; Zemlyanskaya et al., 2021).

8 Practical Applications and Future Research Directions

8.1 Implications for breeding drought-tolerant rice

The development of drought-tolerant rice is crucial for ensuring food security in regions facing water scarcity. The identification of drought-responsive genes and QTLs provides valuable genetic resources for breeding programs. For instance, the study on HuHan2B demonstrated the importance of DEGs in enhancing drought resistance through transcript regulation (Wei et al., 2016). Similarly, the identification of QTLs associated with drought tolerance traits in Vietnamese rice landraces highlights the potential for marker-assisted selection in breeding programs (Hoang et al., 2019). The introgression of elite alleles, such as those found in *OsLG3*, can significantly improve drought tolerance in rice (Xiong et al., 2018). Using CRISPR/Cas9 gene-editing technology, specific genes in the rice genome can be precisely modified to cultivate rice varieties with enhanced drought resistance. The application of this technology not only improves the precision of breeding but also shortens the breeding cycle, providing strong support for the widespread promotion and application of drought-resistant rice. These findings underscore the importance of leveraging genetic diversity and advanced breeding techniques to develop drought-tolerant rice varieties.

8.2 Potential biotechnological applications

Biotechnological approaches offer promising avenues for enhancing drought tolerance in rice. The use of transgenic techniques to overexpress drought-responsive genes, such as TFs and protein kinases, has shown potential in improving drought tolerance (Hadiarto and Tran, 2021). The identification of natural variations in promoter regions, as seen with *OsLG3*, can be exploited to develop transgenic lines with enhanced drought resistance (Xiong et al., 2018).

Additionally, the use of GWAS and gene expression profiling can identify candidate genes and pathways involved in drought response, providing targets for genetic engineering (Gaballah et al., 2020; Hao et al., 2022). By using gene-editing technologies such as CRISPR/Cas9, researchers have successfully knocked out or modified key genes affecting rice yield and quality. By precisely regulating the synthesis and signaling pathways of plant hormones, scientists can cultivate rice varieties with higher yield and better quality. Meanwhile, using RNA interference technology, specific inhibition of pathogen gene expression can enhance rice resistance to certain fungal diseases. Through genetic engineering methods, scientists have successfully increased the content of certain important nutrients in rice, such as β-carotene (precursor of vitamin A) and iron. The integration of these biotechnological tools with traditional breeding methods can accelerate the development of drought-tolerant rice varieties.

8.3 Future research directions and emerging technologies

Future research should focus on the following aspects to further improve our understanding and ability to breed drought-tolerant rice: study of the functional role of identified DEGs and QTLs in drought tolerance through gene knockout and overexpression (Solis et al., 2018), and explore the role of epigenetic modifications in regulating drought-responsive genes and their heritability. In terms of systems biology, systems biology methods are used to model the complex regulatory networks involved in drought response and identify key regulatory nodes, and CRISPR/Cas9 is used to enhance drought tolerance by introducing beneficial alleles and removing harmful alleles. Developing a high-throughput phenotyping platform to accurately measure drought tolerance traits and facilitate large-scale screening of populations for breeding (Gaballah et al., 2020). Combining climate models with breeding programs to predict the performance scenarios of drought-tolerant varieties in future climates (Yu et al., 2012). By addressing these research directions and leveraging emerging technologies, we can make significant progress in developing drought-tolerant rice to ensure sustainable rice production in the face of global climate change.

9 Concluding Remarks

The research on transcriptional regulation and gene networks in rice under water deficit conditions has revealed several critical insights. Genome-wide analyses have identified significant MTAs specific to limited water conditions, highlighting genes such as *OsOFP2*, *Dlf1*, and *OsMADS56* that are crucial for adaptation to water shortages. EGRINs have been shown to coordinate gene expression in response to water deficit, involving 113 TFs and 4052 target genes. Additionally, the DEGs under drought stress has been found to be highly tissue- and stage- specific, with significant roles played by TFs and unique cis-elements. These findings underscore the complexity and specificity of the genetic responses to water deficit in rice.

The integration of various genomic and transcriptomic approaches has been pivotal in advancing our understanding of water deficit responses in rice. By combining GWAS with transcriptional analyses, researchers have been able to identify key genetic loci and regulatory networks that confer drought resistance. The use of EGRINs has further elucidated the dynamic regulatory mechanisms that control gene expression under stress conditions. Such integrated approaches enable a holistic view of the plant's response, facilitating the identification of candidate genes and regulatory elements that can be targeted for breeding programs aimed at improving drought tolerance.

The study of transcriptional regulation and gene networks in rice under water deficit conditions has provided valuable insights into the genetic and molecular mechanisms underlying drought resistance. The identification of specific MTAs, TFs, and regulatory networks highlights the potential for developing more resilient rice varieties through targeted breeding and genetic engineering. Future research should continue to leverage integrated genomic and transcriptomic approaches to further unravel the complex interactions between genes and environmental stressors, ultimately contributing to sustainable rice production in the face of increasing water scarcity.

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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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