

The Regulation Network and Mechanism of Transcriptome in Rice Response to Cold Stress: microRNA Centered

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Abstract Cold stress is a significant abiotic factor that adversely affects rice (*Oryza sativa*) growth and productivity. This systematic review aims to elucidate the integrated regulatory networks of microRNAs (miRNAs) and transcriptome in rice response to cold stress and their underlying mechanisms. Various studies have demonstrated that miRNAs play crucial roles in regulating gene expression under cold stress by targeting specific mRNAs for degradation or translational repression. For instance, miRNAs such as osa-miR1846a/b-5p have been implicated in chilling stress tolerance by regulating genes involved in wax synthesis. Additionally, transcription factors like OsMyb4 and OsERF096 have been shown to orchestrate complex transcriptional networks that enhance cold tolerance through various signaling pathways, including those mediated by abscisic acid (ABA), jasmonic acid (JA), and reactive oxygen species (ROS). The integration of miRNA and transcriptome sequencing has revealed that miRNAs and their target genes are involved in diverse biological processes such as protein biosynthesis, redox processes, and chloroplast development, which are crucial for cold stress response. This review synthesizes current knowledge on the miRNA-mediated regulatory networks and their interaction with the transcriptome, providing insights into the molecular mechanisms underlying rice cold stress tolerance.

Keywords MicroRNAs ; Cold stress; Rice (*Oryza sativa*); Transcriptome; Regulatory networks

1 Introduction

Cold stress is a significant abiotic factor that adversely affects rice (*Oryza sativa* L.) growth and productivity, particularly in tropical and subtropical regions. Exposure to low temperatures can lead to various physiological and developmental issues, including leaf discoloration, reduced tiller numbers, delayed heading, and spikelet sterility, ultimately resulting in decreased yields (Jiang et al., 2019). Cold stress can also cause tissue damage and stunted growth, further limiting rice production (Lv et al., 2010). Given the importance of rice as a staple food crop for a large portion of the global population, understanding the mechanisms underlying cold stress response is crucial for developing cold-tolerant rice varieties.

MiRNAs are small non-coding RNAs that play critical roles in regulating gene expression at the post-transcriptional level. They are involved in various biological processes, including stress responses (Liu et al., 2008; Lv et al., 2010; Barrera-Figueroa et al., 2012). The identification and characterization of stress-responsive miRNAs have provided insights into how plants, including rice, adapt to environmental stresses (Barrera-Figueroa et al., 2012; Jiang et al., 2019). Integrated regulatory networks involving miRNAs and transcriptome data offer a comprehensive understanding of the molecular mechanisms underlying stress responses. These networks can reveal the interactions between miRNAs and their target genes, as well as the broader regulatory pathways involved in stress adaptation (Cohen and Leach, 2019; Liu et al., 2020; Mazurier et al., 2022). By studying these integrated networks, researchers can identify key regulatory elements and potential targets for genetic manipulation to enhance stress tolerance in rice.

The main purpose of this systematic study is to identify and characterize miRNAs involved in cold response in rice: This involves profiling miRNAs that are differentially expressed under cold stress conditions and validating

their expression patterns. Elucidate the regulatory networks involving miRNAs and their target genes: By integrating small RNA sequencing (sRNA-seq) and RNA sequencing (RNA-seq) data, the study aims to construct comprehensive regulatory networks that highlight the interactions between miRNAs and their target genes during cold stress. Understand the molecular mechanisms underlying cold stress response: The study seeks to uncover the pathways and processes regulated by miRNAs and their target genes, providing insights into the molecular basis of cold tolerance in rice. Identify potential genetic targets for improving cold tolerance: By pinpointing key regulatory elements and genes involved in cold stress response, the study aims to provide valuable information for breeding programs aimed at developing cold-tolerant rice varieties.

This systematic review will synthesize current knowledge on the integrated regulatory networks of miRNAs and transcriptome in rice response to cold stress, offering a foundation for future research and practical applications in rice improvement.

2 Conceptual Framework of Integrated Regulatory Networks

2.1 Definition and components of regulatory networks

Regulatory networks in biological systems are intricate frameworks that govern gene expression and cellular functions. These networks are composed of various elements, including transcription factors (TFs), miRNAs, and their target genes. TFs are proteins that bind to specific DNA sequences to regulate the transcription of genetic information from DNA to mRNA. MiRNAs, on the other hand, are small non-coding RNAs that post-transcriptionally regulate gene expression by binding to complementary sequences on target mRNAs, leading to their degradation or translational repression (Le et al., 2013; Guo et al., 2016; Sharma et al., 2019). The interplay between these components forms a complex regulatory network that ensures precise control over gene expression and cellular responses to environmental stimuli.

2.2 Significance of integrating MiRNAs and transcriptome data

Integrating miRNA and transcriptome data is crucial for a comprehensive understanding of gene regulatory mechanisms, especially under stress conditions such as cold stress in rice. MiRNAs play a pivotal role in modulating gene expression in response to abiotic stresses by targeting specific mRNAs for degradation or translational repression (Baldrich et al., 2015; Balyan et al., 2017; Mazurier et al., 2022). By combining miRNA and transcriptome data, researchers can identify miRNA/mRNA target pairs and elucidate the regulatory networks involved in stress responses. This integrative approach allows for the identification of key regulatory nodes and pathways, providing insights into the molecular mechanisms underlying stress tolerance and enabling the development of strategies to enhance crop resilience (Gosline et al., 2015; Sharma et al., 2019; Mazurier et al., 2022).

2.3 Methodological approaches for network construction

Constructing integrated regulatory networks involves several methodological approaches that leverage high-throughput sequencing technologies and computational tools. One common approach is the use of sRNA-seq and RNA-seq to profile miRNA and mRNA expression levels, respectively, under different conditions (Gosline et al., 2015; Balyan et al., 2017; Mazurier et al., 2022). Degradome sequencing is also employed to identify miRNA cleavage sites on target mRNAs, providing direct evidence of miRNA-target interactions (Meng et al., 2011; Baldrich et al., 2015). Computational methods, such as Bayesian network structure learning and network motif finding algorithms, are used to infer the regulatory relationships between miRNAs, TFs, and mRNAs from the expression data (Le et al., 2013; Guo et al., 2016). Additionally, integrating epigenetic data, such as histone modifications, can further refine the regulatory network by revealing the transcriptional and post-transcriptional regulatory layers (Gosline et al., 2015). These comprehensive approaches enable the construction of detailed and accurate regulatory networks that capture the complexity of gene regulation in response to environmental stresses.

By integrating miRNA and transcriptome data, researchers can gain a holistic view of the regulatory networks that govern plant responses to cold stress, ultimately contributing to the development of stress-tolerant rice varieties.

3 MiRNAs in Rice Cold Stress Response

3.1 Identification and characterization of cold-responsive MiRNAs

MiRNAs are small non-coding RNAs that play crucial roles in regulating gene expression in response to various stresses, including cold stress. In rice, several studies have identified cold-responsive miRNAs using different high-throughput sequencing techniques. For instance, a study identified 18 cold-responsive miRNAs in rice using microarrays, revealing that most of these miRNAs were down-regulated under cold stress conditions (Lv et al., 2010). Another study utilized high-throughput sequencing to identify 265 known mature miRNAs and 41 new miRNAs in wild banana, with differential expression analysis showing that some miRNAs were specific for cold treated responses (Liu et al., 2018). These findings highlight the dynamic nature of miRNA expression in response to cold stress and underscore the importance of miRNAs in the cold stress response in rice.

3.2 Functional roles of key MiRNAs

The functional roles of key miRNAs in rice under cold stress have been elucidated through various studies. For example, *osa-miR1320* has been shown to target the ERF transcription factor *OsERF096*, which negatively regulates cold stress tolerance via JA-mediated signaling pathways (Sun et al., 2022). Overexpression of *miR1320* enhances cold tolerance, while its knockdown reduces cold tolerance, indicating its critical role in cold stress response. Additionally, *miR396b* in *Poncirus trifoliata* has been found to regulate cold tolerance by targeting the ACC oxidase gene, thereby modulating ethylene-polyamine homeostasis and enhancing ROS scavenging (Zhang et al., 2016). These studies demonstrate that specific miRNAs play pivotal roles in modulating key regulatory pathways and stress responses in rice under cold stress.

3.3 Regulatory mechanisms of MiRNAs in cold stress

The regulatory mechanisms of miRNAs in cold stress involve complex interactions with their target genes and associated signaling pathways. For instance, *miR1320* regulates cold tolerance by targeting *OsERF096*, which in turn affects JA-mediated signaling pathways and the expression of DREB genes involved in cold stress response (Sun et al., 2022). Similarly, *miR396b* modulates cold tolerance by targeting the ACC oxidase gene, leading to reduced ethylene synthesis and enhanced polyamine synthesis, which helps in ROS scavenging and stress mitigation (Zhang et al., 2016). Furthermore, the presence of hormone-responsive elements in the upstream regions of cold-responsive miRNAs suggests that hormones play a significant role in the miRNA-mediated defense system in rice (Lv et al., 2010). These regulatory mechanisms highlight the intricate network of miRNA-target interactions and their crucial roles in orchestrating the cold stress response in rice.

4 Transcriptome Dynamics under Cold Stress

4.1 Global transcriptomic changes in response to cold stress

Cold stress induces significant changes in the transcriptome of rice, affecting a wide array of genes and pathways. Transcriptomic analyses have revealed that cold stress leads to the differential expression of numerous genes involved in various biological processes. For instance, the integration of miRNA and transcriptome sequencing in wild rice has shown that chilling stress results in the differential expression of miRNAs and their target genes, which are involved in protein biosynthesis, redox processes, photosynthesis, and chloroplast development (Zhao et al., 2022). Additionally, the overexpression of the *OsMyb4* transcription factor (TF) in transgenic rice has been shown to alter the complexity of the transcriptional network, enhancing stress tolerance through the regulation of antioxidant capacity and metabolic processes (Park et al., 2010).

4.2 Identification of cold-responsive genes and pathways

Several studies have identified specific genes and pathways that are responsive to cold stress in rice. For example, the *miR1320-OsERF096* module has been identified as a key regulator of cold tolerance, with *miR1320* targeting the ERF TF *OsERF096*, which in turn modulates hormone content and signaling pathways (Sun et al., 2022). Another study identified 18 cold-responsive miRNAs in rice, with members of the *miR-167* and *miR-319* families showing similar expression profiles under cold stress (Lv et al., 2010). Furthermore, the *OsmiR156-OsSPL3-OsWRKY71* regulatory pathway has been shown to enhance cold stress tolerance by modulating the expression of stress-response genes (Zhou and Tang, 2018).

4.3 Temporal and spatial expression patterns

The temporal and spatial expression patterns of cold-responsive genes and miRNAs are crucial for understanding the regulatory mechanisms underlying cold stress tolerance. For instance, the expression of miR1320 and its target OsERF096 exhibits opposite patterns in different tissues and under cold stress conditions, indicating a tightly regulated response to cold stress (Sun et al., 2022). Similarly, the expression of miRNAs and their target genes in wild rice varies between different genotypes, suggesting that the regulation of miRNAs plays a significant role in the phenotypic variation of chilling tolerance (Zhao et al., 2022). Additionally, the cold-responsive transcription factors identified in both Arabidopsis and rice show distinct expression patterns, with some being more responsive in rice due to its larger genome size and different adaptation strategies (Maryan et al., 2023).

The transcriptome dynamics under cold stress in rice involve complex regulatory networks of miRNAs and TFs that modulate the expression of a wide range of genes and pathways. These findings provide valuable insights into the molecular mechanisms of cold stress tolerance and highlight potential targets for developing cold-tolerant rice varieties.

5 Integration of MiRNAs and Transcriptome Data

5.1 Computational approaches for data integration

The integration of miRNA and transcriptome data is crucial for understanding the complex regulatory networks in rice under cold stress. Computational approaches play a pivotal role in this integration by leveraging high-throughput sequencing data and advanced bioinformatics tools. One such approach involves the use of deep sequencing of small RNA libraries combined with degradome analyses to identify miRNA/target gene pairs and their regulatory networks (Baldrich et al., 2015). Additionally, network frameworks that systematically integrate various high-throughput datasets, such as CHIP-Seq and RNA-Seq, have been developed to construct integrated regulatory networks. These networks include TF to gene, TF to miRNA, and miRNA to gene interactions, providing a comprehensive view of multi-level regulation (Cheng et al., 2011). Mathematical modeling further aids in dissecting the role of miRNAs in gene regulatory networks, elucidating key features such as miRNA-mediated feedback and feedforward loops, and the competition for shared miRNAs (Lai et al., 2016).

5.2 Construction of MiRNAs-gene regulatory networks

Constructing miRNA-gene regulatory networks involves identifying miRNAs and their target genes, followed by the integration of these interactions into a cohesive network. High-throughput sequencing technologies, such as sRNA-seq and RNA-seq, enable the detection of miRNAs and their corresponding mRNA targets. For instance, integrative analyses of sRNA and mRNA sequencing data have identified miRNA/mRNA target pairs with discordant expression patterns, revealing regulatory networks involved in cold response (Mazurier et al., 2022). Additionally, the construction of integrated regulatory networks can be enhanced by incorporating other types of data, such as protein-protein interactions and TF binding profiles, to provide a more detailed and accurate representation of the regulatory landscape (Cheng et al., 2011). Tools like IReNA (Integrated Regulatory Network Analysis) further facilitate the construction of modular regulatory networks, identifying key regulatory factors and significant regulatory relationships among modules (Jiang et al., 2021).

5.3 Validation and functional analysis of integrated networks

Validation and functional analysis of integrated miRNA-gene regulatory networks are essential to confirm the predicted interactions and understand their biological significance. Experimental validation techniques, such as qRT-PCR and reporter assays, are commonly used to verify miRNA-target interactions. Functional analysis involves examining the roles of these interactions in specific biological processes, such as cold stress response. For example, miRNA-mediated regulation of genes involved in hormone signaling and cross-talk among hormone pathways has been shown to play a significant role in rice immunity (Baldrich et al., 2015). Additionally, the integration of miRNA and conserved peptide upstream open reading frame (CPuORF) functions suggests novel regulatory networks that could be crucial for plant stress responses (Baldrich et al., 2015). Computational tools and mathematical models also contribute to the functional analysis by predicting the behavior of regulatory networks and identifying key regulatory motifs, such as feedforward loops, that are over-represented in the network (Cheng et al., 2011; Lai et al., 2016).

6 Case Studies of Integrated Networks in Cold Stress Response

6.1 Specific MiRNA-target interactions and their functional implications

MiRNAs play crucial roles in the regulation of gene expression under cold stress in rice. One notable example is Osa-miR1320, which targets the ERF TF OsERF096. Under cold stress, the expression of miR1320 decreases, leading to an increase in OsERF096 levels. Overexpression of miR1320 enhances cold tolerance, while its knockdown reduces it. OsERF096 negatively regulates cold stress tolerance by modifying hormone content and signaling pathways, particularly the JA-mediated signaling pathway (Figure 1) (Sun et al., 2022). Another study identified 18 cold-responsive miRNAs in rice, with most being down-regulated under cold stress. These miRNAs, including members of the miR-167 and miR-319 families, regulate stress responses by cleaving mRNAs and are involved in hormone signaling pathways (Lv et al., 2010).

6.2 Network motifs and modules in cold stress response

Network motifs and modules are essential for understanding the complex regulatory networks in cold stress response. In rice, miRNAs and TFs form composite feedback loops, which are network motifs that provide high information flow for coordinated regulation. For instance, a study on *Caenorhabditis elegans* revealed that miRNA-TF feedback loops occur more frequently than expected by chance, suggesting their importance in regulatory networks (Martinez et al., 2008). In rice, similar motifs likely exist, where miRNAs and TFs interact to regulate stress responses. Additionally, the integration of miRNA and lncRNA interactions forms complex regulatory networks. For example, in wheat, lncRNAs and miRNAs interact to form ceRNA networks, which play significant roles in cold stress response by regulating antioxidant systems and hormone pathways (Lu et al., 2020).

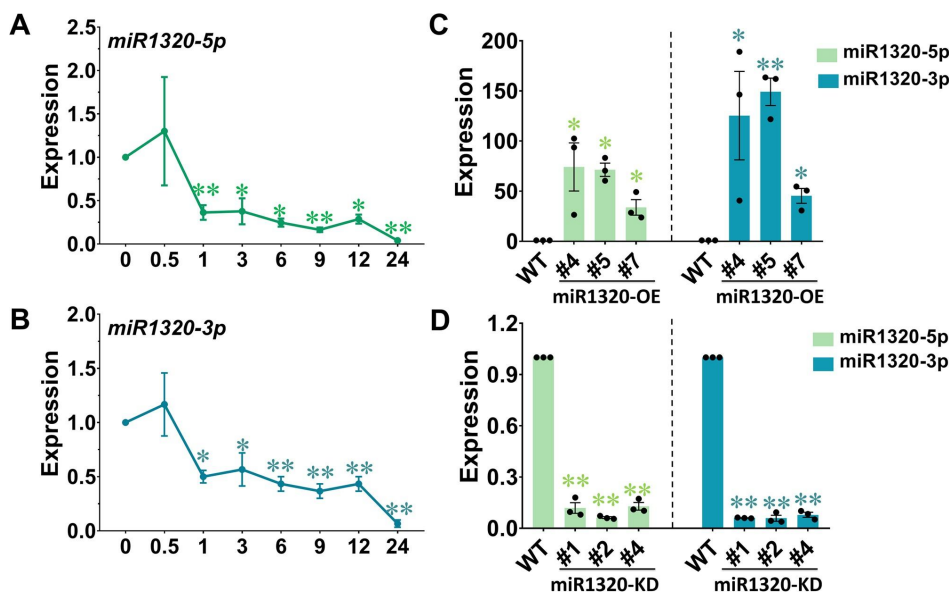


Figure 1 Expression of miR1320 under cold stress and in different transgenic lines (Adopted from Sun et al., 2022)

Image caption: A and B, Expression profiles of miR1320-5p (A) and miR1320-3p (B) under cold stress. C and D, miR1320 expression detection in miR1320-OE (C) and miR1320-KD (D) lines. Three biological replicates and three technical repeats were performed. Data are means \pm SE (n = 3). *P < 0.05; **P < 0.01 by Student's t test (Adopted from Sun et al., 2022)

6.3 Comparative analysis of regulatory networks in different rice varieties

Comparative analysis of regulatory networks in different rice varieties reveals distinct responses to cold stress. For instance, the expression of Osa-miR820 varies between salt-susceptible and salt-tolerant rice varieties under stress conditions. This miRNA is up-regulated in leaf tissues but down-regulated in root tissues under salt stress, indicating a common regulatory scheme with variations in miRNAs and target transcript levels (Sharma et al., 2015). Another study on tropical Asian rice cultivars identified Environmental Gene Regulatory Influence Networks (EGRINs) that coordinate gene expression in response to environmental signals, including cold stress. These networks involve multiple layers of regulation and distinct regulatory roles for TFs, such as heat shock factors, which connect abiotic stress responses to the circadian clock (Wilkins et al., 2016).

The integrated regulatory networks of miRNAs and transcriptome in rice response to cold stress involve specific miRNA-target interactions, network motifs and modules, and comparative analysis across different rice varieties. These networks play crucial roles in modulating gene expression and enhancing cold tolerance through complex regulatory mechanisms.

7 Experimental Approaches for Network Analysis

7.1 High-throughput sequencing technologies

High-throughput sequencing technologies have revolutionized the study of gene regulatory networks by enabling comprehensive profiling of various molecular components. Next-generation sequencing (NGS) technologies, such as RNA-Seq and sRNA-Seq, allow for the detection of weakly expressed transcripts and the identification of regulatory RNAs, including miRNAs (Mazurier et al., 2022). These technologies facilitate the construction of integrated regulatory networks by providing detailed expression profiles of miRNAs and their target genes under different stress conditions (Liu et al., 2020; Mazurier et al., 2022). For instance, in *Pisum sativum*, sRNA-Seq and RNA-Seq were used to identify miRNA/mRNA target pairs involved in cold response, highlighting the role of miRNAs in antioxidative and multi-stress defense mechanisms (Mazurier et al., 2022).

7.2 Functional genomics tools

Functional genomics tools are essential for elucidating the roles of miRNAs within gene regulatory networks. These tools include bioinformatics pipelines, high-throughput target identification platforms, and functional target validation methodologies (Steinkraus et al., 2016). For example, the integration of mRNA and miRNA expression data can reveal the regulatory control exerted by miRNAs on their target genes, as demonstrated in studies on mouse liver gene regulatory networks (Su et al., 2011). Additionally, the development of protocols for systematic assembling and integration of genomics data, such as miRNA-target interactions (MTIs), enables the construction of comprehensive miRNA regulatory maps (Zorc and Kunej, 2015). These integrative approaches help in understanding the complex interactions and regulatory mechanisms at play in response to environmental stresses.

7.3 Integrative multi-omics strategies

Integrative multi-omics strategies combine data from various omics layers, such as genomics, transcriptomics, proteomics, and metabolomics, to provide a holistic view of the molecular networks involved in stress responses (Qin et al., 2016; Yang et al., 2021). These strategies involve the use of bioinformatics tools to integrate multi-dimensional data, generating novel insights into system-level gene regulations (Qin et al., 2016). For instance, multi-omics approaches have been applied to study the regulatory networks in crops like rice, elucidating the relationships between genomes and phenotypes under specific environmental conditions (Yang et al., 2021). The integration of different omics data layers allows for the construction of models that predict complex traits and enhance our understanding of molecular regulator networks for crop improvement (Wörheide et al., 2021; Yang et al., 2021).

The combination of high-throughput sequencing technologies, functional genomics tools, and integrative multi-omics strategies provides a powerful framework for analyzing the integrated regulatory networks of miRNAs and the transcriptome in rice response to cold stress. These approaches enable the identification of key regulatory elements and their interactions, facilitating the development of targeted breeding programs to improve crop resilience and productivity.

8 Applications of Integrated Regulatory Networks

8.1 Implications for breeding cold-tolerant rice varieties

The integration of miRNA and transcriptome sequencing has revealed significant insights into the regulatory mechanisms underlying cold stress tolerance in rice. For instance, the study on the chromosome single segment substitution line (CSSL) DC90, which exhibits a chilling tolerance phenotype, identified differentially expressed miRNAs (DEMs) and their target genes that are involved in various biological processes such as protein biosynthesis, redox processes, and chloroplast development (Zhao et al., 2022). This knowledge can be leveraged in breeding programs to select for rice varieties with enhanced cold tolerance by targeting specific miRNAs and their regulatory networks.

Moreover, the identification of stress-specific miRNA-TF-gene networks in Arabidopsis under cold stress provides a structural perspective that can be applied to rice. This comprehensive regulatory information can accelerate the development of cold-tolerant rice varieties by highlighting potential targets for genetic manipulation (Sharma et al., 2019). Additionally, the role of miRNAs in regulating plant responses to abiotic stresses, including cold, underscores their potential as novel targets for engineering stress-tolerant crop varieties (Figure 2) (Chaudhary et al., 2021).

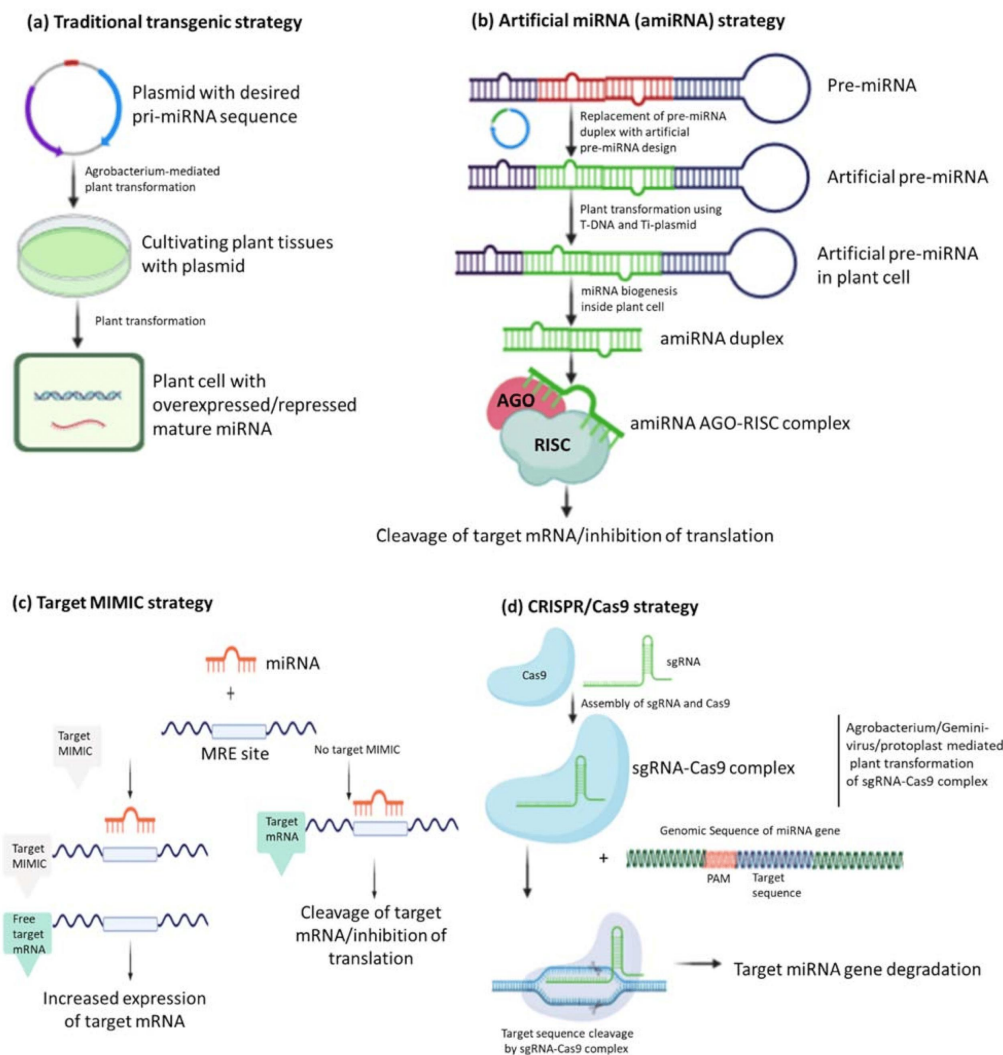


Figure 2 Overview of miRNA-based strategies for crop improvement (Adopted from Chaudhary et al., 2021)

8.2 Potential for genetic engineering and crop improvement

Genetic engineering approaches can exploit the regulatory roles of miRNAs to improve rice tolerance to cold stress. For example, CRISPR/Cas9 editing of target genes of key DEMs in a chilling-tolerant rice variety demonstrated the importance of specific miRNA-target gene pairs in mediating chilling stress tolerance (Zhao et al., 2022). This highlights the potential of using genome editing tools to manipulate miRNA-target interactions for crop improvement.

Furthermore, the application of miRNA gene resources in improving agronomic traits in rice, such as stress tolerance, has been well-documented. miRNAs are involved in various biological processes and can significantly affect plant traits, making them valuable targets for genetic improvement (Zheng and Qu, 2015). The identification of miRNA-mediated regulatory networks in drought-tolerant rice cultivars also provides insights into the dynamic interplay of miRNAs in stress responses, which can be harnessed for developing cold-tolerant varieties (Balyan et al., 2017).

8.3 Translational research and field applications

Translational research aims to bridge the gap between laboratory findings and field applications. The regulatory networks of miRNAs and their target genes identified in rice under cold stress conditions can inform the development of practical strategies for enhancing cold tolerance in rice. For instance, the negative regulatory effects of miR2871b on cold and salt stress tolerance in transgenic rice plants suggest that manipulating the expression of specific miRNAs can improve stress resilience (Yang et al., 2023).

Additionally, multi-omics analyses, including small RNA, transcriptome, and degradome sequencing, provide a comprehensive understanding of the regulatory networks involved in stress responses. This integrated approach can facilitate the identification of key regulatory elements and pathways that can be targeted for crop improvement (Liu et al., 2020). The profiling of miRNA expression domains under stress conditions further supports the potential for developing stress-tolerant rice varieties through targeted manipulation of miRNA expression (Sharma et al., 2015).

The integration of miRNA and transcriptome data offers valuable insights into the regulatory networks governing rice responses to cold stress. These findings have significant implications for breeding, genetic engineering, and translational research aimed at developing cold-tolerant rice varieties.

9 Future Perspectives and Challenges

9.1 Emerging trends in network biology

The study of integrated regulatory networks involving miRNAs and transcriptomes in rice under cold stress has revealed several emerging trends in network biology. One significant trend is the use of high-throughput sequencing technologies, such as RNA-seq and sRNA-seq, to uncover complex regulatory networks. These technologies have enabled the identification of miRNA/mRNA target pairs and the construction of co-expression networks, which are crucial for understanding the molecular mechanisms underlying cold stress responses in rice (Lv et al., 2010; Mazurier et al., 2022; Maryan et al., 2023). Additionally, the integration of multiple genome-scale measurements, such as transcriptome data and chromatin accessibility, has facilitated the inference of Environmental Gene Regulatory Influence Networks (EGRINs), which coordinate gene expression in response to environmental signals (Wilkins et al., 2016). This integrative approach is becoming increasingly important in network biology, as it allows for a more comprehensive understanding of the regulatory interactions and their functional implications.

9.2 Challenges in data integration and interpretation

Despite the advancements in high-throughput sequencing and integrative analysis, several challenges remain in the integration and interpretation of data. One major challenge is the complexity of the regulatory networks, which involve multiple layers of regulation, including transcriptional, post-transcriptional, and epigenetic mechanisms (Cheng et al., 2011; Baldrich et al., 2015; Sharma et al., 2019). The sheer volume of data generated by high-throughput technologies can be overwhelming, making it difficult to identify key regulatory interactions and their functional significance. Additionally, the variability in experimental conditions and the inherent differences between plant species can complicate the comparison and integration of data from different studies (Wang et al., 2019; Maryan et al., 2023). Another challenge is the accurate prediction of miRNA targets and the validation of these interactions, which often require extensive experimental validation (Lv et al., 2010; Sharma et al., 2015). Addressing these challenges will require the development of more sophisticated computational tools and experimental techniques to improve the accuracy and reliability of data integration and interpretation.

9.3 Future research directions

Future research in the field of integrated regulatory networks in rice response to cold stress should focus on several key areas. First, there is a need for more comprehensive and high-resolution datasets that capture the dynamic changes in gene expression and regulatory interactions over time and under different stress conditions (Wilkins et al., 2016; Mazurier et al., 2022). This will enable a more detailed understanding of the temporal and spatial dynamics of regulatory networks. Second, the development of advanced computational models and

algorithms for network inference and data integration will be crucial for unraveling the complexity of these networks (Cheng et al., 2011; Sharma et al., 2019). These models should incorporate multiple layers of regulation and account for the interactions between different regulatory elements, such as miRNAs, TFs, and chromatin modifications. Third, experimental validation of predicted regulatory interactions and the functional characterization of key regulatory elements will be essential for translating the findings from network biology into practical applications, such as the development of cold-tolerant rice varieties (Baldrich et al., 2015; Sharma et al., 2015; Balyan et al., 2017). Finally, comparative studies between different plant species and stress conditions will provide valuable insights into the conserved and species-specific regulatory mechanisms, which can inform the development of more resilient crops (Wang et al., 2019; Maryan et al., 2023).

By addressing these future research directions, we can enhance our understanding of the integrated regulatory networks in rice response to cold stress and develop effective strategies for improving crop resilience to environmental stresses.

10 Concluding Remarks

The integrated regulatory networks of miRNAs and transcriptome in rice response to cold stress reveal a complex interplay of genetic and epigenetic mechanisms. Several studies have identified specific miRNAs and their target genes that play crucial roles in modulating rice's response to cold stress. For instance, miR2871b has been shown to negatively regulate cold stress tolerance by increasing ROS levels and decreasing antioxidant enzyme activities. Additionally, miRNAs such as miR-167, miR-319, and miR-171 exhibit diverse expression patterns under cold stress, indicating their significant roles in stress response. The identification of cold-responsive TFs and their co-expression networks further elucidates the regulatory mechanisms at play. Moreover, the discovery of novel miRNAs and their stress-regulated expression in rice inflorescences highlights the importance of miRNAs in reproductive tissues under cold stress.

Understanding the regulatory networks of miRNAs and transcriptome in rice under cold stress has significant implications for agricultural practices. The identification of specific miRNAs and their target genes provides potential biomarkers for developing cold-tolerant rice varieties. For example, manipulating the expression of miR2871b could enhance cold stress tolerance in rice, thereby improving crop resilience and yield in cold-prone regions. Additionally, the knowledge of cold-responsive TFs and their regulatory networks can be harnessed to engineer rice plants with enhanced stress tolerance through genetic modification or selective breeding. The integration of miRNA and transcriptome data can also inform precision agriculture practices, enabling the development of targeted interventions to mitigate the adverse effects of cold stress on rice production.

The systematic review of integrated regulatory networks of miRNAs and transcriptome in rice response to cold stress underscores the complexity and significance of these regulatory mechanisms. The insights gained from these studies provide a foundation for future research aimed at enhancing cold stress tolerance in rice. By leveraging the identified miRNAs, TFs, and their regulatory networks, researchers and agricultural practitioners can develop innovative strategies to improve rice resilience and productivity in the face of climate change. Continued exploration of these regulatory networks will be crucial for advancing our understanding of plant stress responses and for ensuring global food security.

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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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