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Dynamic Changes and Biological Significance of MicroRNA Expression Profiles in Rice under Cold Stress

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Abstract Cold stress is a significant abiotic factor that adversely affects rice (*Oryza sativa*) growth and productivity. This study investigates the dynamic changes and biological significance of microRNA (miRNA) expression profiles in rice under cold stress. Utilizing high-throughput techniques such as microarrays and stem-loop reverse transcription quantitative PCR (ST-RT qPCR), researchers identified several miRNAs that exhibit differential expression in response to cold stress. Notably, miR1320, miR319, and miR156 were found to play crucial roles in enhancing cold tolerance by targeting specific transcription factors and modulating stress-responsive genes. For instance, miR1320 targets the ERF transcription factor OsERF096, which is involved in jasmonic acid (JA)-mediated signaling pathways, while miR319 targets OsPCF6 and OsTCP21, contributing to the regulation of cold stress-responsive genes such as *DREB1A/B/C* and *TPP1/2*. Additionally, miR156 enhances cold tolerance by targeting OsSPL3, which in turn regulates the expression of OsWRKY71 and other stress-related transcription factors. These findings underscore the importance of miRNAs in the complex regulatory networks that govern rice's response to cold stress, providing valuable insights for developing cold-tolerant rice varieties.

Keywords Cold stress; MicroRNA; Rice (*Oryza sativa*); Gene regulation; Abiotic stress tolerance

1 Introduction

Rice (*Oryza sativa* L.) is a staple food for more than half of the world's population, making it a critical crop for global food security. However, rice cultivation is highly sensitive to environmental stresses, particularly cold stress, which can significantly impact growth, development, and yield. Cold stress, characterized by low temperatures, can lead to physiological and biochemical changes in rice plants, affecting processes such as photosynthesis, nutrient uptake, and cellular metabolism. Understanding the mechanisms underlying rice's response to cold stress is essential for developing strategies to enhance cold tolerance and ensure stable rice production under adverse climatic conditions.

MiRNAs are small, non-coding RNAs that play crucial roles in regulating gene expression at the post-transcriptional level. They are involved in various biological processes, including development, growth, and stress responses. In plants, miRNAs have been shown to modulate the expression of genes involved in stress tolerance, making them key players in the adaptation to environmental challenges. For instance, miR319 has been identified as a significant regulator in rice, influencing leaf morphogenesis and enhancing cold tolerance by modulating the expression of target genes such as *OsPCF5* and *OsPCF8* (Yang et al., 2013). MiR396b has been identified can actively regulates cold tolerance by reducing 1-aminocyclopropane-1-carboxylic acid oxidase (ACO) transcription levels (Zhang et al., 2016). And, miR535 has been confirmed to inhibit early growth of seedlings under cold stress due to its overexpression. It negatively regulates rice cold stress response by targeting three SPL genes *OsSPL14/11/4* and mediating the CBF-mediated cold signaling pathway (Mingzhe et al., 2022). Additionally, miRNAs are known to participate in the regulation of hormone metabolism, carbohydrate metabolic pathways, and cell division, which are critical for plant development and stress responses (Ahmed et al., 2020;

Pan et al., 2021). The differential expression of miRNAs under various stress conditions, including cold stress, highlights their potential as targets for genetic manipulation to improve stress tolerance in rice.

The objective of this study is to investigate the dynamic changes and biological significance of miRNA expression profiles in rice under cold stress. By analyzing the expression patterns of miRNAs and their target genes, this study aims to elucidate the molecular mechanisms underlying rice's response to cold stress. Specifically, the study seeks to identify miRNAs that are differentially expressed in rice under cold stress conditions, characterize the functional roles of these miRNAs in regulating stress-responsive genes, and explore the potential of miRNAs as biomarkers or targets for enhancing cold tolerance in rice through genetic engineering. By achieving these objectives, the study aims to contribute to the development of rice varieties with improved cold tolerance, thereby ensuring stable rice production in regions prone to low-temperature stress.

2 Overview of MiRNA Biogenesis and Function

2.1 MiRNA biogenesis pathway

MiRNAs are small, endogenous RNA molecules that play crucial roles in the regulation of gene expression. The biogenesis of miRNAs begins in the nucleus, where primary miRNAs (pri-miRNAs) are transcribed by RNA polymerase II. These pri-miRNAs are then processed by the microprocessor complex, which includes the RNase III enzyme Dicer-like 1 (DCL1) and the double-stranded RNA-binding protein HYPONASTIC LEAVES 1 (HYL1), to produce precursor miRNAs (pre-miRNAs). The pre-miRNAs are subsequently exported to the cytoplasm by the exportin-5 homolog HASTY, where they are further processed by DCL1 to generate mature miRNA duplexes. One strand of the duplex, the guide strand, is incorporated into the RNA-induced silencing complex (RISC), while the other strand, the passenger strand, is typically degraded (Figure 1) (Manavella et al., 2019).

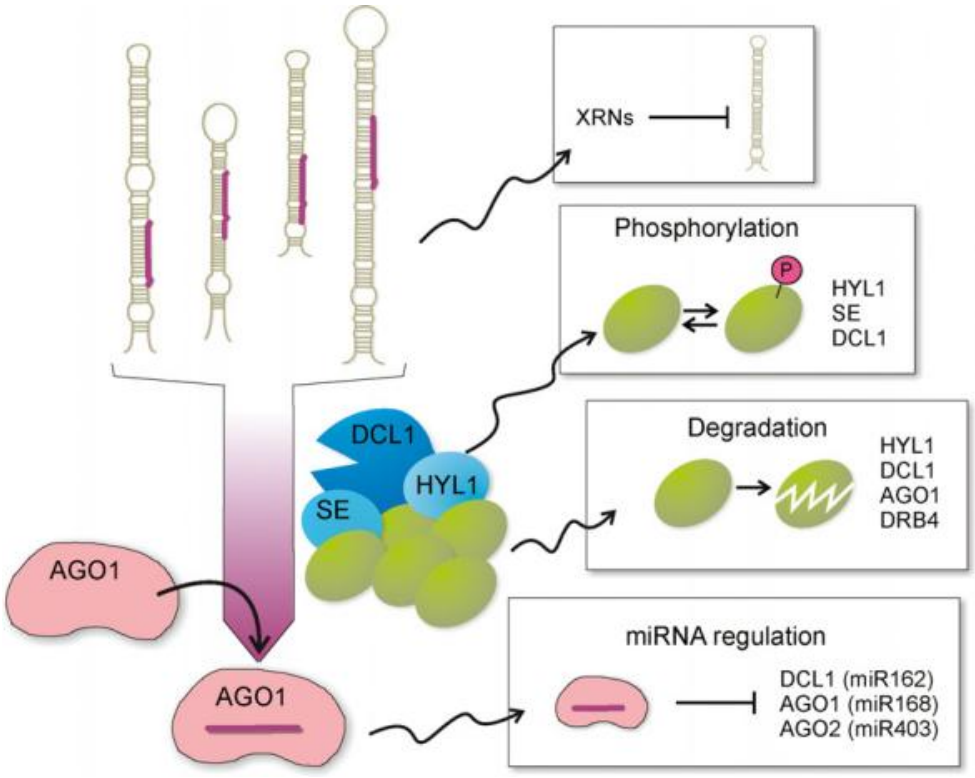

Figure 1 Scheme showing the miRNA biogenesis pathway and regulatory steps (Adopted from Manavella et al., 2019)

Image caption: Precursors with many different structures are processed by a multi-protein complex that harbors DCL1, HYL1 and SE as core components. This complex cuts the precursors to release the miRNA that becomes incorporated in an AGO protein, generally AGO1. Regulatory steps include the control of XRN activity, protein phosphorylation/dephosphorylation, protein stability and feedback regulation by miRNAs targeting DCL1 and AGO1 (Adopted from Manavella et al., 2019)

2.2 Mechanisms of miRNA action

Once incorporated into the RISC, the guide strand of the miRNA directs the complex to complementary target mRNAs, leading to either mRNA cleavage or translational repression. The choice between these two mechanisms depends on the degree of complementarity between the miRNA and its target. Perfect or near-perfect complementarity usually results in mRNA cleavage, while partial complementarity leads to translational repression. In plants, miRNAs predominantly guide the cleavage of target mRNAs, which is a critical mechanism for regulating gene expression during development and stress responses (Manavella et al., 2019).

2.3 Role of miRNAs in plant development and stress responses

MiRNAs are integral to various aspects of plant development and stress responses. They regulate key developmental processes by targeting transcription factors and other regulatory genes. For instance, miRNAs such as miR156, miR159, and miR160 are involved in the regulation of flowering time, leaf development, and root architecture (Fard et al., 2017).

In response to abiotic stresses, miRNAs modulate the expression of genes involved in stress tolerance. For example, during cold stress, specific miRNAs such as miR167 and miR319 are down-regulated, while others like miR171 show diverse expression patterns, indicating their role in the cold stress response (Fard et al., 2017). Similarly, miRNAs like miR395 are up-regulated under sulfate starvation, highlighting their role in nutrient homeostasis (Baldrich et al., 2015).

Moreover, miRNAs are also involved in the regulation of hormonal pathways, which are crucial for stress adaptation. For instance, miR399 is known to mediate cross-talk between cytokinin and phosphorus deficiency signaling, while miR394 is involved in auxin-mediated regulation of sulfur homeostasis during sulfur deficiency (Grewal et al., 2018). These regulatory roles underscore the importance of miRNAs in maintaining plant homeostasis under varying environmental conditions.

In summary, miRNAs are pivotal in orchestrating plant development and stress responses by fine-tuning gene expression through mRNA cleavage and translational repression. Their ability to regulate a wide array of genes makes them essential for plant adaptation to both biotic and abiotic stresses (Baldrich et al., 2015; Zhang, 2015; Fard et al., 2017; Grewal et al., 2018; Dubey et al., 2019).

3 Cold Stress in Rice: Physiological and Molecular Responses

3.1 Physiological effects of cold stress on rice

Cold stress significantly impacts rice, a crop of tropical origin, by affecting its growth, development, and productivity. Physiological responses to cold stress include alterations in photosynthesis, water use efficiency, and gas exchange parameters. For instance, tropical japonica genotypes such as Secano do Brazil and Cypress exhibit a greater reduction in these parameters compared to temperate japonica genotypes like Nipponbare and M202 (Freitas et al., 2019). Additionally, cold stress induces changes in chlorophyll fluorescence, electrolyte leakage, and the accumulation of reactive oxygen species (ROS) and malondialdehyde (MDA), which are indicators of oxidative stress (Zhang et al., 2016; Liu et al., 2018). The accumulation of antioxidants and osmolytes, such as phenolic compounds and proline, helps maintain cellular homeostasis under cold conditions (Freitas et al., 2019). Proteomic analyses have revealed that proteins involved in antioxidative defense, protein biosynthesis, and cell wall biosynthesis are upregulated in response to cold stress (Cui et al., 2005). Comparative metabolomic studies have highlighted the role of ROS in mediating the cold stress response and recovery, with significant biochemical changes observed in antioxidation-related compounds and amino acids (Pradhan et al., 2019; Kong et al., 2020).

3.2 Molecular mechanisms of cold stress tolerance

At the molecular level, rice plants activate a series of genetic and biochemical pathways to cope with cold stress. Differential gene expression plays a crucial role, with genes such as *OsGH3-2*, *OsSRO1a*, *OsZFP245*, and *OsTPP1* showing significant changes in expression levels under cold conditions (Figure 2) (Freitas et al., 2019). Transcription factors like OsDREB2A, AP2/ERF-ERF, NAC, WRKY, MYB, C2H2, and bHLH are also involved

in regulating the cold stress response (Zhao et al., 2015; Lv et al., 2019). The glutathione system, involving enzymes like glutathione peroxidase (GPX) and glutathione S-transferase (GST), serves as a primary ROS scavenger, mitigating oxidative damage (Zhao et al., 2015). Additionally, hormone signaling pathways, particularly those involving abscisic acid (ABA), play a dominant role in the cold stress response (Zhao et al., 2015). Furthermore, quantitative trait loci (QTLs) associated with cold tolerance have been identified through linkage and association mapping, providing valuable insights for breeding cold-tolerant rice varieties (Lv et al., 2019). Transcriptome analyses have identified differentially expressed genes and pathways involved in cold tolerance, including those related to energy metabolism, signal transduction, and photosynthesis (Hsu and Hsu, 2019). The physiological and molecular responses of rice to cold stress involve complex interactions between genetic, biochemical, and physiological pathways. Understanding these mechanisms is crucial for developing strategies to enhance cold tolerance in rice, thereby ensuring stable production in the face of climatic challenges.

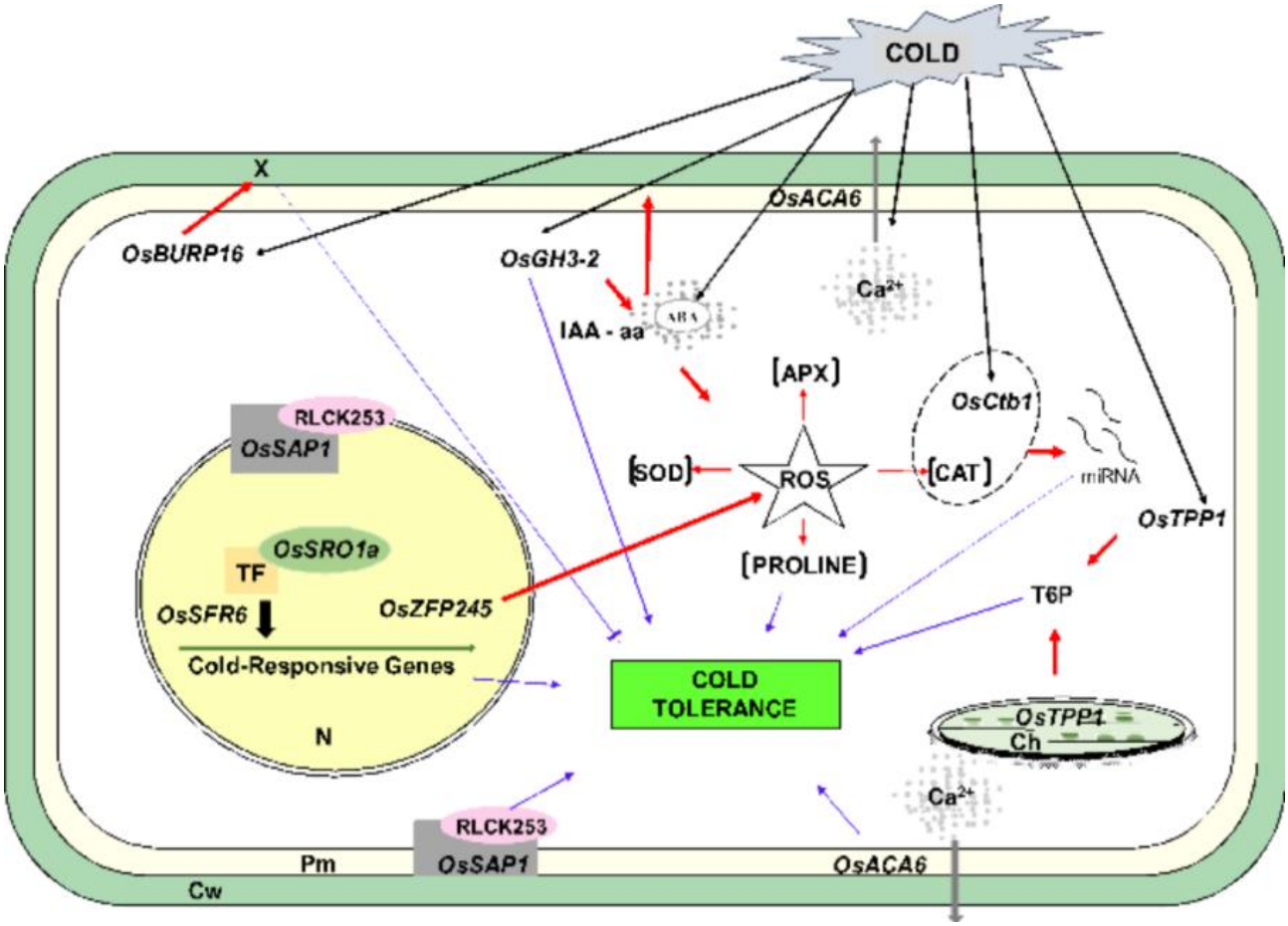

Figure 2 Model displaying mechanisms of tolerance to low temperatures in rice (Adopted from Freitas et al., 2019)

Image caption: The regulatory cascade indicates the perception and induction of damage in response to low temperatures, the response in gene expression changes to the stress treatment, as well as the induction of biochemical responses leading to low temperature tolerance, with an increase in concentration due to the presence of ROS. Abbreviations shown indicate the changes in components affected. Pm: Plasma membrane; Cw: Cell wall; Ch: Chloroplast; N: Nucleus; Grey arrow: Calcium efflux Black arrows: Cold perception; X: Degradation of pectin caused by increased polygalacturonase induced by increased expression of *OsBURP16*; Red arrow: Induction; Blue arrow: Induction of cold tolerance; Between brackets: Increase in concentration due to ROS. Dotted: Association of *Ctb1* and CAT for miRNA induction. Upward arrows: Increased concentration due to induction of ROS; X: Association of *Ctb1* and CAT for miRNA induction. The regulatory cascade of perception and induction of damage in response to low temperatures, and response of genes to the stress treatment, as well as the induction of biochemical responses leading to tolerance to low temperatures, leading from an increased concentration of ROS. This image represents a model of the molecular mechanisms involved in cold tolerance in rice. It illustrates how different genes, proteins, and signaling pathways interact to enhance cold tolerance (Adopted from Freitas et al., 2019)

4 MiRNA Expression Profiling in Rice under Cold Stress

4.1 Experimental approaches for miRNA profiling

MiRNA profiling in rice under cold stress involves several experimental approaches to identify and quantify miRNAs. One common method is the use of microarrays, which can detect the expression levels of multiple miRNAs simultaneously. For instance, a study identified 18 cold-responsive miRNAs in rice using microarrays, revealing that most were down-regulated under cold stress (Lv et al., 2010). Another approach is the one-tube ST-RT qPCR, which has been optimized for high-throughput expression profiling of miRNAs in rice. This method is accurate, convenient, and time-saving, making it suitable for quantifying miRNAs under various stress conditions, including cold stress (Shen et al., 2010; Sharma et al., 2015). Additionally, miRNA arrays have been used to profile miRNA expression in rice accessions exposed to different stress conditions, including cold stress, to identify differentially expressed miRNAs (Sharma et al., 2015).

4.2 High-throughput sequencing and bioinformatics analysis

High-throughput sequencing technologies, such as Illumina deep sequencing, have revolutionized miRNA profiling by providing comprehensive and detailed expression data. For example, deep sequencing of small RNA libraries constructed from rice plants under different stress conditions, including cold stress, identified numerous miRNAs and their expression patterns (Pritchard et al., 2012). This method allows for the discovery of novel miRNAs and the assessment of their regulation under stress conditions. In another study, high-throughput sequencing of rice inflorescences under cold stress identified 227 miRNAs, including 70 novel ones, and revealed their differential expression (Peng et al., 2011). Bioinformatics analysis plays a crucial role in processing the vast amount of sequencing data, predicting miRNA targets, and understanding the regulatory networks involved in stress responses. For instance, bioinformatics tools were used to predict and categorize key miRNAs and their target genes involved in arsenic stress in rice (Sharma et al., 2015).

4.3 Validation techniques for miRNA expression

Validation of miRNA expression is essential to confirm the results obtained from high-throughput profiling methods. Quantitative reverse transcription PCR (qRT-PCR) is a widely used technique for validating miRNA expression levels. This method was employed to validate the differential expression of miRNAs identified in rice under various stress conditions, including cold stress (Dubey et al., 2019). Another validation technique is the use of 5’ rapid amplification of cDNA ends (5’ RACE) to confirm miRNA-target interactions. For example, two miRNA target pairs were validated using 5’ RACE, showing opposite expression profiles under cold stress (Lv et al., 2010). Additionally, small RNA gel blotting can be used to validate miRNA expression, although it is less convenient and time-consuming compared to qRT-PCR (Shen et al., 2010). MiRNA expression profiling in rice under cold stress involves a combination of experimental approaches, high-throughput sequencing, bioinformatics analysis, and validation techniques. These methods collectively provide a comprehensive understanding of the dynamic changes and biological significance of miRNA expression profiles in rice under cold stress.

5 Dynamic Changes in MiRNA Expression under Cold Stress

5.1 Differentially expressed miRNAs in response to cold stress

Cold stress significantly impacts the expression of various miRNAs in rice. In a study profiling cold-stress-responsive miRNAs, 18 miRNAs were identified, with most showing down-regulation under cold conditions. Notably, members of the miR-167 and miR-319 families exhibited similar expression profiles, while miR-171 family members displayed diverse patterns (Wen et al., 2016). Additionally, miR1320 was found to be down-regulated under cold stress, and its overexpression increased cold tolerance, whereas its knockdown reduced cold tolerance (Sun et al., 2022). These findings highlight the critical role of miRNAs in modulating rice’s response to cold stress.

5.2 Temporal and spatial patterns of miRNA expression

The expression of miRNAs in response to cold stress is not uniform across different tissues and developmental stages. For instance, miR820 is present in all tissues, but it is abundantly expressed in mature leaf and root tissues.

Moreover, the expression of Osa-miR820 was found to be up-regulated in leaf tissues but down-regulated in root tissues under salt, high temperature, and drought stress, suggesting a potential similar regulatory mechanism under cold stress (Sharma et al., 2015). These temporal and spatial expression patterns indicate that miRNAs may have distinct roles in different tissues and at various stages of development in response to cold stress.

5.3 Case studies of specific miRNAs involved in cold stress response

MiR1320 plays a pivotal role in cold stress response by targeting the AP2/ERF transcription factor OsERF096. Overexpression of miR1320 enhances cold tolerance, while its knockdown reduces it. OsERF096 negatively regulates cold stress tolerance and is involved in the JA-mediated signaling pathway, which is crucial for cold stress response (Wen et al., 2016; Sun et al., 2022).

Members of the miR-167 and miR-319 families were identified as cold-responsive miRNAs, with both families showing similar expression profiles under cold stress. These miRNAs are involved in regulating various stress-related targets, further supporting their role in cold stress response (Lv et al., 2010).

The miR-171 family exhibited diverse expression patterns under cold stress, indicating that different members of this family may have distinct roles in the cold stress response. This diversity in expression suggests a complex regulatory mechanism involving miR-171 in adapting to cold stress (Lv et al., 2010; Yi et al., 2013). The dynamic changes in miRNA expression under cold stress in rice involve a complex interplay of various miRNAs, each contributing to the plant's ability to cope with adverse conditions. Understanding these changes provides valuable insights into the molecular mechanisms underlying cold stress tolerance in rice.

6 Biological Significance of MiRNA-Mediated Regulation under Cold Stress

6.1 Target gene identification and functional annotation

MiRNAs play a crucial role in regulating gene expression under cold stress by targeting specific genes. For instance, miR1320 targets the ERF transcription factor OsERF096, which negatively regulates cold stress tolerance by modifying hormone content and signaling pathways (Jeong et al., 2011). Additionally, miR167 and miR319 families have been identified to show similar expression profiles under cold stress, indicating their potential roles in stress response (Sun et al., 2022). The identification of these target genes and their functional annotation helps in understanding the molecular mechanisms underlying cold stress tolerance in rice.

6.2 MiRNA-target gene interaction networks

The interaction networks between miRNAs and their target genes are complex and involve multiple regulatory pathways. For example, osa-miR167 inhibits shoot growth but activates adventitious root growth by influencing free auxin content during mineral deficiency, which can be extrapolated to cold stress conditions (Grewal et al., 2018). Moreover, miR1320-OsERF096 interaction modulates the JA-mediated cold signaling pathway, highlighting the intricate network of miRNA-target interactions that contribute to cold stress tolerance (Jones-Rhoades and Bartel, 2004; Sun et al., 2022). These networks are essential for coordinating the plant's response to cold stress and ensuring survival and adaptation.

6.3 Functional roles of key miRNAs in cold stress tolerance

Key miRNAs such as miR1320, miR167, and miR319 play significant roles in enhancing cold stress tolerance in rice. Overexpression of miR1320 (miR1320-OE) results in increased cold tolerance, while its knockdown (miR1320-KD) reduces cold tolerance, demonstrating its critical role in cold stress response (Figure 3) (Sun et al., 2022). Similarly, miR167 and miR319 families are involved in regulating developmental and stress responses, further supporting their importance in cold stress tolerance (Campo et al., 2013; Singh et al., 2020). The functional roles of these miRNAs are vital for developing strategies to improve cold stress tolerance in rice through genetic and biotechnological approaches. By understanding the biological significance of miRNA-mediated regulation under cold stress, researchers can develop targeted interventions to enhance cold tolerance in rice, thereby improving crop yield and resilience in adverse environmental conditions.

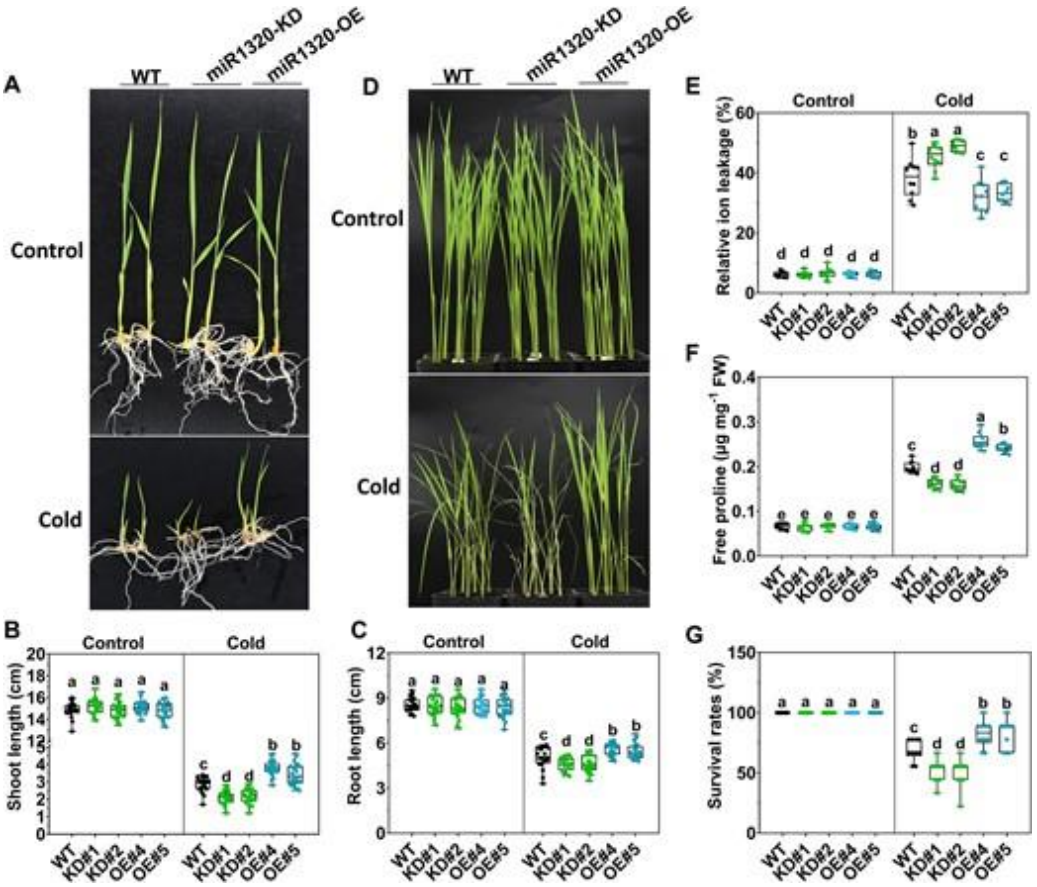

Figure 3 miR1320 positively regulates cold tolerance (Adopted from Sun et al., 2022)

Image caption: A-C, Phenotype (A), shoot length (B), and root length (C) of the WT, miR1 320 KD, and miR1320-OE seedlings during the cold stress test at the early seedling stage. D-G, Phenotype (D), relative ion leakage (E), free proline content (F), and survival rates (G) of the WT, miR1320-KD, and miR1320-OE seedlings during the cold stress test at the three-leaf stage. The horizontal line inside the box represents the median. The box represents the values between first and third quartiles (interquartile range, IQR). Upper or lower whiskers delineate maximum and minimum values, respectively. Different lowercase letters indicate significant difference among different lines (ANOVA test, $P<0.05$) (Adopted from Sun et al., 2022)

7 Integration of MiRNA and Transcriptome Data

7.1 Co-expression analysis of miRNAs and mRNAs

Co-expression analysis of miRNAs and mRNAs is a crucial step in understanding the regulatory mechanisms under cold stress in rice. By examining the expression profiles of both miRNAs and mRNAs, researchers can identify potential regulatory relationships and functional interactions. For instance, a study on rice under various environmental stresses, including cold stress, identified numerous miRNAs that are differentially expressed, suggesting their involvement in stress response pathways (Jeong et al., 2011; Wang et al., 2015). Similarly, another study highlighted the differential expression of miRNAs in rice under chromium stress, which could be extrapolated to cold stress scenarios, indicating the importance of miRNA-mRNA interactions in stress responses (Dubey et al., 2019). These findings underscore the significance of integrating miRNA and mRNA expression data to elucidate the complex regulatory networks in rice under cold stress.

7.2 Regulatory network construction

Constructing regulatory networks involves mapping the interactions between miRNAs and their target mRNAs to understand the underlying biological processes. In rice, miRNAs play a pivotal role in regulating gene expression under stress conditions. For example, the integration of miRNA and mRNA expression data has been used to construct miRNA-mRNA networks that reveal the regulatory mechanisms in response to abiotic stresses (Lv et al., 2010). Additionally, tools like *Oryza* Express facilitate the construction of gene expression networks by

integrating various omics data, providing a comprehensive view of the regulatory interactions in rice (Xu et al., 2018). These networks help in identifying key regulatory nodes and pathways that are crucial for stress adaptation and resilience.

7.3 Insights from integrated omics approaches

Integrated omics approaches, combining data from miRNA, mRNA, and other molecular levels, offer deeper insights into the regulatory mechanisms under cold stress. For instance, a multi-omics analysis in tetraploid wheat under stress conditions revealed the intricate networks of miRNAs and their target genes, providing valuable information on stress adaptation mechanisms (Hamada et al., 2010; Liu et al., 2020). Similarly, integrating miRNA and mRNA expression data in cancer research has shown that such approaches can identify more precise regulatory interactions and potential therapeutic targets (Seo et al., 2017). In rice, these integrated approaches can uncover novel regulatory pathways and key miRNAs involved in cold stress response, ultimately aiding in the development of stress-tolerant rice varieties. By leveraging the power of integrated omics data, researchers can gain a holistic understanding of the dynamic changes in miRNA expression profiles and their biological significance in rice under cold stress. This comprehensive approach not only enhances our knowledge of stress response mechanisms but also paves the way for innovative strategies in crop improvement and stress management.

8 Genetic Engineering and Breeding for Cold Tolerance

8.1 Strategies for manipulating miRNA expression

MiRNAs play a crucial role in regulating plant responses to cold stress. Various strategies have been employed to manipulate miRNA expression to enhance cold tolerance in rice. For instance, overexpression of miR1320 has been shown to increase cold tolerance by targeting the ERF transcription factor OsERF096, which is involved in the JA-mediated signaling pathway (Sun et al., 2022). Similarly, overexpressing miR319 in rice leads to enhanced cold tolerance by targeting genes such as *OsPCF6* and *OsTCP21*, which are involved in active oxygen scavenging and stress-responsive gene expression (Yang et al., 2013; Wang et al., 2014). Conversely, overexpression of miR2871b in Dongxiang wild rice results in decreased cold tolerance, indicating its negative regulatory role in stress responses (Yang et al., 2023).

8.2 Transgenic approaches and genome editing

Transgenic approaches and genome editing techniques, such as CRISPR/Cas9, have been effectively utilized to enhance cold tolerance in rice. For example, the CRISPR/Cas9 system has been used to edit genes like *OsPIN5b*, *GS3*, and *OsMYB30*, resulting in rice mutants with improved cold tolerance and yield (Cui et al., 2015). Additionally, overexpression of miR156 in rice has been shown to increase cold stress tolerance by targeting the SPL3 gene, which regulates the expression of various stress-responsive transcription factors (Zhou and Tang, 2018; Zeng et al., 2020). However, it is important to note that not all miRNA manipulations lead to positive outcomes; for instance, overexpression of miR156k has been found to reduce cold tolerance in rice seedlings (Figure 4) (Hsu and Hsu, 2019; Yue et al., 2020).

8.3 Potential applications in rice breeding programs

The insights gained from manipulating miRNA expression and employing genome editing techniques have significant potential applications in rice breeding programs. By targeting specific miRNAs and their associated genes, breeders can develop rice varieties with enhanced cold tolerance. For example, miR528 has been identified as a potential target for increasing cold stress tolerance by repressing the expression of stress response-related transcription factors (Tang and Thompson, 2019). Similarly, miR535 has been suggested as a genetic editing target for improving drought and salinity stress tolerance, which could be integrated into breeding programs to develop multi-stress tolerant rice varieties (Yue et al., 2020). The integration of these advanced molecular techniques into traditional breeding programs can accelerate the development of rice cultivars that are better equipped to withstand cold stress and other environmental challenges.

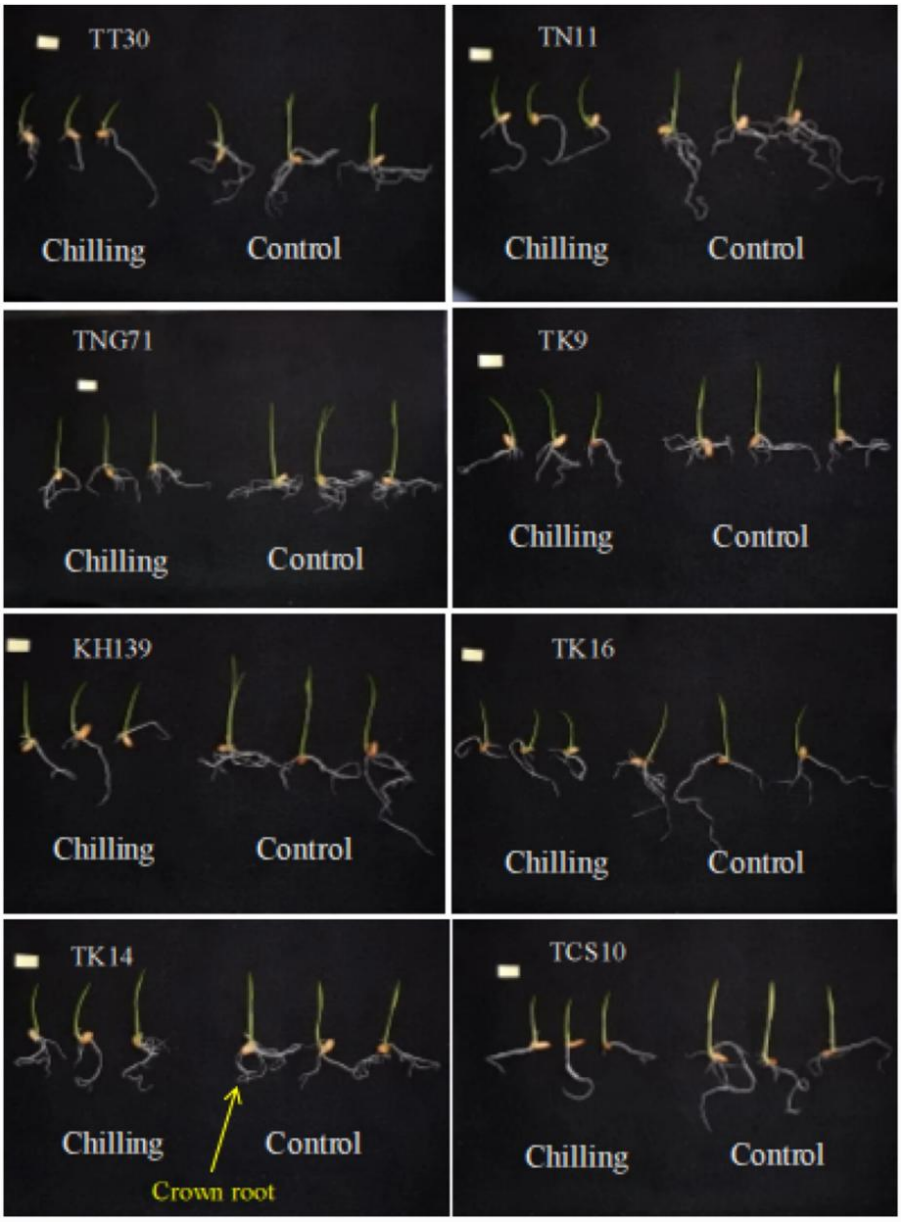

Figure 4 The cold stress response of rice seedlings (Adopted from Hsu and Hsu, 2019)

Image caption: Figure shows a comparison of rice seedlings of different varieties under near-cold stress (labeled "Control") with rice seedlings grown under control conditions (labeled “Control”). These varieties are labeled TT30, TN11, TNG71, TK9, KH139, TK16, TK14 and TCS10. Cold conditions appear to affect seedling growth and development, especially crown roots, as indicated by the yellow arrow in the TK14 variety panel (Adopted from Hsu and Hsu, 2019)

9 Future Perspectives and Challenges

9.1 Emerging technologies in miRNA research

The field of miRNA research in rice, particularly under cold stress, is rapidly evolving with the advent of new technologies. High-throughput sequencing and microarray analyses have significantly advanced our understanding of miRNA expression profiles and their regulatory roles. For instance, deep sequencing of small RNA libraries has enabled the identification of numerous miRNAs and their variants, providing insights into their tissue-specific and stress-responsive expression patterns (Lv et al., 2010; Jeong et al., 2011). Additionally, the development of one-tube ST-RT qPCR has facilitated high-throughput and accurate quantification of miRNAs, making it a valuable tool for profiling miRNA expression under various stress conditions (Kaur et al., 2023). These technologies are expected to further unravel the complex regulatory networks of miRNAs, enhancing our ability to manipulate miRNA pathways for improved stress tolerance in rice.

9.2 Gaps in current knowledge and research directions

Despite significant progress, several gaps remain in our understanding of miRNA-mediated responses to cold stress in rice. One major gap is the limited knowledge of the specific target genes of cold-responsive miRNAs and their precise roles in stress adaptation. While some studies have identified miRNA target pairs and predicted stress-related targets (Lv et al., 2010; Dubey et al., 2015), comprehensive functional validation of these targets is still lacking. Additionally, the dynamic changes in miRNA arm selection under different stress conditions and their biological significance are not fully understood (Hu et al., 2014). Future research should focus on elucidating the molecular mechanisms underlying miRNA-target interactions and the functional roles of miRNAs in cold stress tolerance. Integrating multi-omics approaches, such as transcriptomics, proteomics, and metabolomics, could provide a holistic view of miRNA-regulated stress responses.

9.3 Implications for sustainable rice production

Understanding the dynamic changes and biological significance of miRNA expression profiles in rice under cold stress has profound implications for sustainable rice production. Cold stress is a major abiotic factor that adversely affects rice yields by causing tissue damage and stunting growth (Lv et al., 2010). By identifying and characterizing cold-responsive miRNAs, researchers can develop rice varieties with enhanced cold tolerance, thereby improving crop resilience and productivity. For example, the differential expression of miRNAs in response to cold stress can be leveraged to engineer rice plants with optimized stress response pathways (Lv et al., 2010; Sharma et al., 2015). Moreover, the identification of miRNAs involved in other abiotic stresses, such as drought and salinity, highlights the potential for developing multi-stress tolerant rice varieties (Shen et al., 2010; Zhang et al., 2017). Ultimately, these advancements will contribute to achieving food security and sustainability in rice cultivation under changing climatic conditions.

10 Concluding Remarks

In this study, we investigated the dynamic changes and biological significance of miRNA expression profiles in rice under cold stress. Our findings revealed that cold stress significantly alters the expression of various miRNAs, with most being down-regulated. Notably, members of the miR-167 and miR-319 families exhibited similar expression profiles, while miR-171 family members showed diverse patterns. The miR1320-OsERF096 module was identified as a key regulator of cold tolerance, modulating the JA-mediated signaling pathway. Additionally, miR319 overexpression was found to enhance cold tolerance and impact leaf morphogenesis. We also highlighted the role of hormone-responsive elements in the upstream regions of cold-responsive miRNAs, indicating the importance of hormonal regulation in cold stress responses.

The insights gained from this study have significant implications for crop improvement. The identification of specific miRNAs and their target genes involved in cold stress responses provides potential targets for genetic engineering to enhance cold tolerance in rice. For instance, overexpressing miR319 or manipulating the miR1320-OsERF096 module could be promising strategies to develop rice varieties with improved cold tolerance. Furthermore, understanding the hormone-mediated regulation of miRNAs under cold stress can aid in the development of hormone-based treatments to mitigate the adverse effects of cold stress on rice crops. These approaches could ultimately lead to increased rice yields and stability in regions prone to low-temperature stress.

In this study, we underscores the critical role of miRNAs in regulating rice responses to cold stress. The dynamic changes in miRNA expression profiles and their biological significance provide a deeper understanding of the molecular mechanisms underlying cold tolerance in rice. Future research should focus on validating these findings in field conditions and exploring the potential of miRNA-based genetic modifications for developing cold-tolerant rice varieties. By leveraging the knowledge gained from this study, we can make significant strides in improving rice resilience to cold stress, thereby ensuring food security in the face of climate variability.

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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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