

# MicroRNA: One of the Stress Fighters Plays Crucial Role in Resilience of Rice to Changing Climatic Conditions

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### ABSTRACT

This century is presenting global climate change with significant alterations in environmental conditions, which might affect the growth, development, and survival of several organisms. In turn, such changes affect the availability of food, feed, and fodder for the creatures on the earth. Recurring environmental stresses like heat, drought, cold, flooding, etc. might cause considerable yield losses, challenges to crop husbandry, and concerns for sustainable food security. Modulation in gene expression under stressful conditions is one of the molecular strategies adopted by plants to cope with environmental stresses. MicroRNAs (miRNAs) are known to play important roles in controlling gene expression either through translational repression or due to cleavage of mRNA. Moreover, miRNAs are emerging as newer candidates for modulating developmental processes including productivity/yield as well as responses to stress in plants. Often the targets of miRNAs are transcription factors and the genes associated with stress responses affecting adaptive potential of the plant. A combination of miRNAs (miR160-ARF, miR159-MYB, and miR169-NFYA) was reported to be involved in regulating gene expression under drought in plants. These drought-responsive miRNAs were shown to affect physiological, biochemical, and molecular responses and serving as candidates for genetic manipulation of crop plants for enhanced stress resilience. This review provides insights on miRNA as a stress fighter that plays an important role in resilience to environmental stresses in plants, particularly in rice. miRNA has been reported to control crucial biological processes like respiration, photosynthesis, signalling pathways, senescence, etc., particularly under stressful conditions. Some of the limitations as well as future perspectives of utilizing miRNA-based strategies for improvement have been discussed. These might help understanding the functions of miRNAs, as one of the important components of the gene regulatory network, which will facilitate genetic improvement of crops for multiple stresses and yielding potential.

Keywords: Gene expression, Gene regulation, microRNA, Rice, Environmental stress, Abiotic stress, Drought tolerance, Stress tolerance.

#### **INTRODUCTION**

In the present century of global climate change, environmental factors (abiotic as well as biotic) are adversely affecting the growth, development, and productivity of crop plants. Abiotic stresses including drought, flooding, low light, heat, cold, salinity, etc., resulting from unpredicted climate change, pose significant constraints to crop production and the nutritional quality of the produce [1]. These are emerging as important issues for sustainable global food security [2]. Such adverse environmental factors may disrupt cellular processes by increasing electrolyte leakage, damage to cellular membranes, accumulation of reactive oxygen species (ROS), oxidative damage to plant cells [3]. In contrast, the accrual of osmolytes like soluble sugars and proline plays crucial functions in osmotic adjustment and mitigation of stress in plants [4,5,6].

Currently, rice (*Oryza sativa* L.) meets the calorie requirement of about 70% of Asian populations [7] and it is grown in more than 100 countries over 161 million hectares world over [8].

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More importantly, an additional 120 million tons of rice will require to be produced by the year 2035 to feed the rapidly growing global human population. With a limited possibility of increasing areas under rice cultivation, particularly in the conventional rice growing regions, extra rice needs to be produced on continuously shrinking cultivable lands under the risks imposed by climatic changes [9]. Moreover, ensuring the nutritional quality of food materials would be another challenging task while increasing productivity [2,10]. Importantly, rice production can also be increased by cultivating it in unconventional areas in the rice-consuming regions, for which the development of various abiotic/biotic stress-tolerant high-yielding rice cultivars will be one of the prerequisites. However, due to the increasing severity of climatic changes, it is speculated that rice crops will face diverse/multiple abiotic stresses in fields in future.

Abiotic stresses, particularly drought, heat, and cold, are known to adversely affect growth, development, productivity, and yield of rice crop, as it is a water-loving, warm-season crop [7,11]. Various abiotic stresses may cause poor germination/stand establishment, stunted growth of plants, and mortality of seedling leading to yield losses of up to 40% [12]. Stresses like drought, heat, and cold disrupt cellular processes due to increased electrolyte leakage, damage to cellular membranes, accumulation of ROS, and oxidative damage to plants [13,14]. In contrast, the accumulation of osmolytes like soluble sugars and free proline play crucial roles in osmotic adjustment and stress mitigation. Moreover, regulatory networks at transcriptional, post-transcriptional as well as translational level play crucial roles in tolerance to stresses. Several genes including those for transcription factors (TFs) and kinases play important roles in tolerance to stresses like drought, heat, and low temperature.

The functions of microRNA (miRNA) in growth/development, vital physiological processes, phytohormonal signaling/ crosstalk, defense responses, as well as responses to different abiotic stresses are well reported [15]. However, the precise action/function of miRNAs under different/multiple stresses in rice has not yet been fully understood. Therefore, the need of the day is to unravel the miRNAs involved in tolerance to abiotic stresses in rice to develop stress-resilient rice varieties, particularly for the regions prone to low-temperature, salinity, flooding, rain-fed agriculture, etc., which has become necessary for sustainable food production/security in rice-consuming countries.

miRNAs are reported to play crucial roles in controling several biological processes like respiration, energy metabolism, hormone signaling, abiotic stresses, and senescence. Increased expression of miR408 in the leaf of Arabidopsis was reported to regulate SPL7 and HY5 involved in copper and light signaling [16]. In addition, regulation at epigenetic, transcription, and post-transcription (epitranscriptomic) levels play crucial roles in tolerance to abiotic stresses [1720]. Several stress-responsive genes including those coding for TFs and kinases play important roles in abiotic stress tolerance [2123]. The importance/involvement of miRNAs in plant growth and development is continuously being reported [15,19,24,25]. Thus, the need of the day is to understand the functions of miRNAs associated with tolerance to environmental stresses in crop plants like rice towards the development of stress-resilient cultivars, particularly for the regions prone to multiple environmental stresses, for sustainable food production.

A study on cold responses of rice demonstrated the involvement of miRNA (miR535) in stress tolerance [26]. To analyze the mechanism of cold tolerance, miR535 overexpressing transgenic lines, and wild-type rice plants were exposed to low temperature stress followed by a recovery period, wherein survival of the plants and their performance at the physiological level including ion leakage, accumulation of reactive oxygen species (ROS), and osmotic imbalance were assessed. Coldinduced expression of miR535 was reported to repress seedling growth in the transgenic line under cold stress, compared to that in the non-transgenic plants. Overexpression of miR535 was also reported to increase ROS accumulation and higher malondialdehyde levels. Activity of the enzymes involved in scavenging of ROS (SOD, POD, and CAT) was recorded to be significantly reduced in transgenic lines under stress. In terms of osmotic regulation, the transgenic lines showed an accumulation of soluble sugars than that in the non-transgenic (wild-type) plants under stress. Thus, miR535 negatively regulates tolerance to cold stress in rice by targeting genes of SPL family influencing the CBF-mediated signaling pathway. Overexpression of miR535 was reported to downregulate the expression of OsCBF1, OsCBF2, and OsCBF3 genes as well as the downstream cold-responsive genes like OsRAB16A and OsRAB16B. Additionally, miR535 also modulated the expression of SPL genes, with significant downregulation of OsSPL14, OsSPL11, and OsSPL4, in the transgenic lines under stress. A recent study on drought tolerance in tice [25] reported 68 novel miRNAs up-regulated in addition to 43 down-regulated novel miRNAs in the panicle of a drought tolerant Nagina 22 rice cultivar on terminal drought stress. Moreover, certain known miRNAs (e.g., osamiR396, osamiR166, osamiR167, osamiR156, and osamiR812) were recorded to be expressed differentially in the root of rice [19].

#### miRNA as a stress fighter

MiRNA, a short (1822 nt) non-coding RNA, mediates gene silencing by targeting specific mRNA via its degradation or translational repression [21,24]. miRNAs are crucial posttranscriptional regulators to fine-tune gene expression in response to abiotic stresses. Understanding such regulatory mechanisms is important for developing stress-tolerant crops through genetic engineering/biotechnological approaches. The c-repeat binding factor (CBF) pathway has been one of the wellcharacterized mechanisms for plants to respond to chilling stress [27]. CBFs are induced on exposure to low temperatures which activate downstream cold-responsive (COR) genes through binding to CRT/DRE motifs in the promoter, which results in the accumulation of defensive proteins and metabolites. This pathway has been utilized to improve stress tolerance by modulating biochemical and physiological processes. The miRNAs playing a role in stress tolerance by controlling specific genes/pathways are being identified using high throughput sequencing technologies. Several miRNAs including miR396 [28], miR319 [29], miR394 [30], miR397 [31], miR408 [32], miR535 [26], miR1320 [14], and miR1868 [12] have been characterized for their role in fighting against cold/chilling stress in rice. These miRNAs target different genes and pathways to modulate plant's responses to chilling stress. With just about 800 miRNAs submitted so far in the miRBase dataset expressing in different rice tissues under different environmental states (estimated to be only about 2-3% of miRNAs encrypted by the genome) [18], the number and role of miRNAs involved in stress tolerance are yet elusive, necessitating further research in this area to elucidate their function and regulatory mechanism.

Among the differentially expressed miRNAs in the root of rice, 12 novel and 18 known miRNAs were reported to be expressed exclusively in the root of Nagina 22 on terminal drought stress, while zero novels and only two known miRNAs were reported to be expressed exclusively in the root of IR 64 [19]. The target gene of majority of the miRNAs was reported to be the droughtresponsive TFs which plays vital role in auxin signaling, root development, flower, grain development, and phytohormonecrosstalk. Among the 111 novel miRNAs reported to express in the panicle of Nagina 22 on terminal drought stress, 68 were recorded to be up-regulated while 43 were observed to be down-regulated under stress. Moreover, 31 novel miRNAs observed to be up-regulated in the panicle of Nagina 22 were recorded to be down-regulated in the case of IR 64 under stress. In addition, four novel miRNAs reported to be down-regulated in the panicle of Nagina 22 were up-regulated in the case of IR 64 on terminal drought stress. Similarly, the targets of the differentially expressed novel miRNAs were identified to be TFs and stress-responsive genes involved in cellular, metabolic, and developmental processes, as well as those associated with responses to abiotic stresses, programmed cell death under drought stress [25].

#### Cold tolerance in rice

A study explored the role of miR1320 utilizing a transgenic approach, wherein overexpression as well as knockdown of miRNA in rice was performed [14]. Overexpressing lines showed significantly upregulated expression of the miRNA under stress with reduced accumulation of ROS, and ion leakage while higher activities of antioxidant enzymes and better survival of the plant. The target of miR1320 (*OsERF096*) plays an important role in the jasmonic acid (JA) signaling pathway.

Functional analysis of miR1320 indicated that it targets *OsERF096* through cleavage of mRNAs. The findings suggest that miR1320 acts as one of the positive regulators for cold tolerance in rice by controling JA signaling pathway (Figure 1).



Figure 1. The key miRNAs involved in controling cold/chilling tolerance in rice.

A recent study determined the function of miR1868 in cold stress tolerance in rice [12]. While miR1868 overexpressing transgenic rice lines showed increased ion leakage and decreased cold tolerance (lower survival), the knockdown lines showed reduced ion leakage, improved cold tolerance, and a better survival rate compared to non-transgenic plants. Increased activity of the enzyme (POD, SOD, and CAT) involved in antioxidant activities in the knockdown lines (while lower activity in overexpressing lines) indicated its role in regulating ROS scavenging under cold stress. Lower MDA content (an indicator of oxidative damage) in knockdown lines confirmed its function. RNA-seq analysis revealed that miR1868 modulates the expression of eleven different genes associated with starch and sucrose metabolism under stress. Thus, the findings suggest that miR1868 functions as a negative controler of cold tolerance in rice by affecting ROS scavenging and carbohydrate metabolic pathways. These studies highlight the crucial function of miRNAs in regulating responses to cold stress in rice. While miR535 and miR1868 act as negative regulators (adversely affecting cold tolerance by modulating ROS accumulation and oxidative damage), miR1320 enhances cold tolerance by promoting antioxidant enzyme activity and reducing cell death under stress. These findings demonstrate the potential of utilizing miRNAs towards the improvement of cold tolerance and productivity of rice under low-temperature.

#### Drought tolerance in rice

In the panicle of rice, 148 novel miRNAs were reported to target 409 genes associated with stress responses on the imposition of terminal drought [25]. The target genes of these miRNAs were reported to be the stress-responsive TFs like, ARF, NB-ARC, WRKY, GRAS, bZIP, ERF, GLTP, GRF, TFIIS, and LRR. Several of the important genes associated with drought stress tolerance are targeted by the known/novel miRNAs which included inositol 1,3,4-triphosphate 5/6-kinase (known for drought and salt tolerance), *OsLAC2* (associated with biosynthesis of lignin as well as stress tolerance), *OsPIP1* (affecting water uptake and water use efficiency), and *GRAS* (involved in drought and oxidative stress tolerance), vacuolar monosaccharide symporter 1. The up-regulated expression of some of the known miRNAs (miR160, miR159, miR156) [33,34], and novel miRNAs

(osa-novel\_miR\_019, osa-novel\_miR\_012, osa-novel\_miR\_009, osa-novel\_miR\_008, osa-novel\_miR\_006,) in the panicle of Nagina 22 (that were reported to be down-regulated in the panicle of IR 64) on reproductive stage drought was held responsible for better performance of Nagina 22 under stress [25]. A miRNA (osa-novel\_miR\_036) targeting WRKY71 was reported for its important role in flowering under stress [35]. Moreover, 7.9-fold up-regulated expression of osa-novel\_miR\_007 in the panicle of Nagina 22 (while 3-fold down-regulated expression in the panicle of IR 64) with down-regulation of its target gene (LOC\_0s01g46600) was reported to play important role in seed development under stress in the panicle of Nagina 22 [25].

Likewise, a total of 1847 stress-associated genes were reported to be targeted by miRNAs in the root of rice on reproductive stage drought [19]. A detailed analysis of the novel and known miRNAs targeted genes indicated TFs like WRKY, NB-ARC, Zinc finger domains, MYM, NAC, Leucine-rich repeat domains, RLCK, GRAS, MATH, bZIP, MAPK, ARFs, HSP, MADS [33,3537], and transporters (glutathione-conjugate transporter, ABC transporters, sugar/inositol transporters, and those for sodium, potassium, etc.) to be the important targets. The same miRNA targeting multiple genes as well as a certain gene targeted by multiple known and/or novel miRNAs were reported, which confirmed interactions among the mRNAs and miRNAs under stress [25]. Such interactions between miRNA and its target gene(s) are crucial to control the expression of gene(s) associated with stress responses (Figure 2). OsSPL transcription activators, that affect shoot and plant architecture in rice, was reported to be targated by miR156 [19,38]. Similarly, differentially expressed seven members of miR393 and miR159 families was reported to affect horizontal root development, tiller number, stem elongation, floral development under terminal drought stress by controlling OsTIR1 and OsAUX1 [3941].



**Figure 2.** Pictorial presentation of some of the droughtresponsive novel/known miRNAs, targeted genes, and responses on terminal drought stress, as reported in the panicle of the rice cultivar Nagina 22 (modified from [25]).

## Limitations in adopting a miRNA-based strategy for crop improvement

Despite a significant progress made in understanding the mode of action and functions of miRNAs in abiotic/biotic stress

responses, numerous key questions remain unanswered, which need to be answered before miRNA-based strategies can be designed/adopted [15] to enhance various abiotic/biotic stress resiliance in rice [42]. Some of the queries include, but are not limited to, investigations on the following points.

- → What are the mechanisms controlling the biogenesis and stability of stress-responsive miRNAs?
- → How do genetic variations in miRNA genes and their targets contribute to different stress tolerance among various rice genotypes?
- → How do different miRNAs coordinate with each other as well as with other regulatory molecules in mediating appropriate stress responses?
- → What are the miRNAtarget interaction networks under stress, and how do these interaction dynamics change over time/generation?
- → What are the specific interactions between miRNAs and TFs in regulation of stress responses at different developmental stages of rice?
- → How do long non-coding RNAs (lncRNAs) interact with miRNAs and their targets to modulate stress responses in rice?
- → How do miRNAs integrate into signal transduction pathways to regulate stress responses?
- → How do different environmental factors (such as drought and/or salinity) interact with another abiotic stress to affect miRNA expression/function?
- → How do epigenetic modifications (like biogenesis of siRNA/lncRNA, histone modification, and DNA methylation) affect the expression of miRNAs under stress?
- → What would be an effective strategy to utilize the knowledge of miRNAs in crop improvement programs to enhance multiple stress tolerance?

#### **Future perspectives**

Answers to the aforementioned questions would provide deeper insights into the complex regulatory networks controlling stress responses/tolerance, which might facilitate enhancing stress resilience in rice. Investigations on coordination among miRNAs, involvement of micro-peptide (miPEP) [43], and other regulatory molecules (including TFs and lncRNAs) will provide better insights into their role in stress responses. Future research would also need to focus on elucidating the interaction between epigenome (DNA methylome as well as histone modifications) and miRNome in regulating gene expression under simultaneous or subsequent stresses. Moreover, exploring the genetic diversity in miRNA genes across the rice germplasm, including wild rice accessions, might aid in molecular breeding for enhanced tolerance. Finally, integrating the understanding of miRNA with ever-growing molecular tools and techniques [44] would be effective/efficient enough in the development of multiple stress tolerant rice cultivars for stress-prone areas to ensure sustainable food security, particularly in rice-consuming areas.

#### CONCLUSION

MiRNAs are continuously being reported to play crucial function in controling gene expression not only under developmental processes but also under stressful conditions. More importantly, their role in post-transcriptional cum self-catalyzing activities is being deciphered. Many of the miRNAs targeting TFs indicate their function as a regulator of master regulators. miRNAs being one of the stress fighters perform important roles in stress tolerance and emerges as one of the candidate genes for improving responses to abiotic stresses. A better understanding of the action and interactions of miRNAs with other regulatory molecules might help in designing more efficient/effective strategies for the genetic improvement of plants to improve the yielding potential of rice under stress.

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