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Post-Transcriptional Regulation by microRNAs during Drought in Rye

Guiping Zhang, Wei Wang
Institute of Life Sciences, Jiyang College of Zhejiang A&F University, Zhuji, 311800, Zhejiang, China
Corresponding email: wei.wang@jicat.org

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Abstract Rye (*Secale cereale* L.) is renowned for its strong adaptability in marginal environments and is of great significance for ensuring food security and achieving sustainable agriculture in areas with frequent droughts. This study systematically explored the miRNA expression dynamics of rye under drought stress and its mechanism of action in gene silencing, signal integration and stress adaptation. Through high-throughput sequencing technology, multiple differentially expressed mirnas under drought conditions were identified, and their potential target genes were predicted. The results of functional annotation and network analysis indicated that these mirnas were mainly involved in regulating key pathways such as reactive oxygen species (ROS) clearance, abscisic acid (ABA) signaling, and transcriptional regulation related to stress responses. In the case studies of miR398, miR159 and miR166, the research revealed how specific mirNA-target gene interactions affect the physiological characteristics of rye under drought stress, including ROS clearance efficiency, hormone signal regulation and leaf morphology changes, etc. This study not only deepens the understanding of the molecular response mechanism of rye to drought, but also provides new ideas and technical support for mirNa-based functional genomics research and the improvement of stress-resistant crops in the context of climate change.

Keywords Rye; Drought stress; microRNA; Post-transcriptional regulation; Drought resistance mechanism

1 Introduction

Rye (*Secale cereale* L.) has not been a leading grain for a long time, but it has shown astonishing resilience in some difficult growing environments, especially in dry or infertile soil conditions, where it is more resilient than most cereal crops. So, as the global climate becomes increasingly extreme, especially with the frequency and intensity of droughts continuing to rise, rye has naturally been placed back on the key list for research and breeding. It not only plays a complementary role in food security, but also offers a relatively safe option for sustainable agriculture. However, to enable rye to perform better under water shortage conditions, it is necessary to figure out what molecular mechanisms are "supporting" it (Cheng et al., 2022).

In recent years, researchers have increasingly focused their attention on microRNA (miRNA). These small non-coding RNA molecules, which are only about 20 bases in length, can "dictate" a large number of genes after transcription, and they are extremely precise. They not only involve key drought resistance links such as abscisic acid (ABA) signaling, ROS clearance system and root configuration, but also have been confirmed to be involved in the response regulation under drought stress in a variety of plants (Singh et al., 2022). Although these findings mainly come from well-studied crops such as rice and corn, rye and its "distant relatives" - other gramineous plants - also show signs that the regulatory potential of miRNA should not be underestimated (Ferdous et al., 2015; Singroha et al., 2021; Zhang et al., 2022; Zhakypbek et al., 2025). At present, many studies are no longer merely exploring the existence of these small Rnas, but have gone further, taking them as potential entry points for future crop stress-resistant breeding.

This study aims to systematically explore the post-transcriptional regulatory mechanism mediated by miRNA under drought stress in rye, review the current understanding of miRNA biosynthesis and function in plant drought response, summarize the latest research achievements of miRNA in drought response in rye and its related species, and discuss the application of bioinformatics and experimental methods in miRNA target identification and validation. By clarifying the regulatory network related to miRNA, this study aims to provide a scientific basis for future breeding strategies and biotechnology intervention measures to enhance the drought tolerance of rye and other crops, and to ensure global food security under climate change conditions.

2 Physiological and Molecular Responses of Rye to Drought Stress

2.1 Effects of drought stress on rye growth and development

Rye often performs worse than expected in drought years, especially with significant limitations in grain yield, biomass and root development (Bao et al., 2022). Different test results show that the yield reduction can sometimes reach 57%, but there are also varieties that have only decreased by about 14%. In fact, not only the yield, but also multiple developmental stages such as the number of tillers, leaf spreading, and root extension will be disturbed (Kottmann et al., 2016; Makhramova and Urokov, 2024). Some varieties will show the phenomenon of early maturity, and the photosynthetic area will also decrease accordingly. Physiologically, water loss, decreased photosynthetic capacity, and hormonal imbalances (especially the reduction of trans zeaxin nucleosides) all make it difficult for plants to maintain normal growth (Vedenicheva et al., 2024). However, not all rye is the same. Some varieties with special genetic backgrounds can still maintain relatively stable productivity when water is scarce, indicating that there are certain genetic differences in drought resistance.

2.2 Drought-related signal transduction pathways in rye

When rye encounters drought, it does not simply act passively but activates a complete set of signal transduction mechanisms within its body. ABA (abolic acid) is often the earliest mentioned regulatory hormone. Its accumulation can activate a series of genes that respond to drought, such as those encoding serine/threonine protein kinases (Tomita et al., 2021; Movahedi et al., 2023). But in fact, apart from ABA, cytokinin and ROS (reactive oxygen species) are also at play. They regulate growth and defense responses to a certain extent. Some transcription factors, such as WRKY, DREB and NAC, are significantly upregulated under drought conditions, and this expression change can link multiple stress response genes (Cheng et al., 2022). This entire pathway will eventually control a series of processes such as stomatal closure, osmotic regulation, and antioxidant reactions, helping rye alleviate the impact of drought.

2.3 Overview of drought resistance traits and gene expression regulation networks

The drought tolerance of rye is rarely determined by a single gene. It is usually the result of the combined effects of root structure, antioxidant levels and metabolic homeostasis. At the molecular level, researchers have identified thousands of differentially expressed genes and proteins under different conditions, involving pathways such as energy metabolism, lipid synthesis, and signal transduction (Pan et al., 2017; 2018). What regulates these changes is a vast regulatory network. Among them, there are transcription factors and protein kinases, as well as E3 ligases involved in the ubiquitination process. Together, they regulate ABA signaling and its related stress pathways (Wang et al., 2023). These genes and networks are associated with some obvious drought-resistant traits, which can provide a clear direction for the breeding of drought-tolerant rye varieties.

3 Basic Features and Functional Mechanisms of microRNAs

3.1 Biosynthesis and modes of action of microRNAs

The synthesis process of miRNA seems like a complex assembly line, from gene transcription to the loading of mature miRNA into protein complexes, each step is not simple. miRNA usually consists of only 20 to 24 nucleotides and is an endogenous non-coding RNA that plays the role of a regulator in plants. They were initially synthesized into pri-miRNA by RNA polymerase II, and this precursor folds into a stem-ring structure (Zhang and Wang, 2025). DCL1 is the core enzyme involved in the processing. Meanwhile, HYL1 and SERRATE also get involved, gradually processing pri-miRNA into pre-miRNA and then transforming it into mature miRNA/miRNA double-stranded entities. This process all takes place in the cell nucleus. When the mature miRNA is ready, it will be methylated, then loaded into the AGO protein to form the RISC complex, and then transported to the cytoplasm to continue "functioning" (Figure 1) (Wang et al., 2019).

3.2 Post-transcriptional regulatory mechanisms mediated by microRNAs

Once miRNA enters the RISC complex, it does not directly control the transcription of genes. Instead, it "targets" mrnas that are highly complementary to its own sequence, usually making a cut at these targets. After the cut, the mRNA is degraded. Sometimes, it does not cut but blocks the initiation or extension of translation, depending on the type of miRNA. It is worth noting that some mirnas with a length of 22 nucleotides can also trigger a chain reaction of Phasirnas, as if opening up another regulatory channel. This mechanism is particularly important in regulating the developmental process or responding to stress, making the regulation of gene expression more flexible (Dong et al., 2022; Ding and Zhang, 2023; Xu and Chen, 2023).

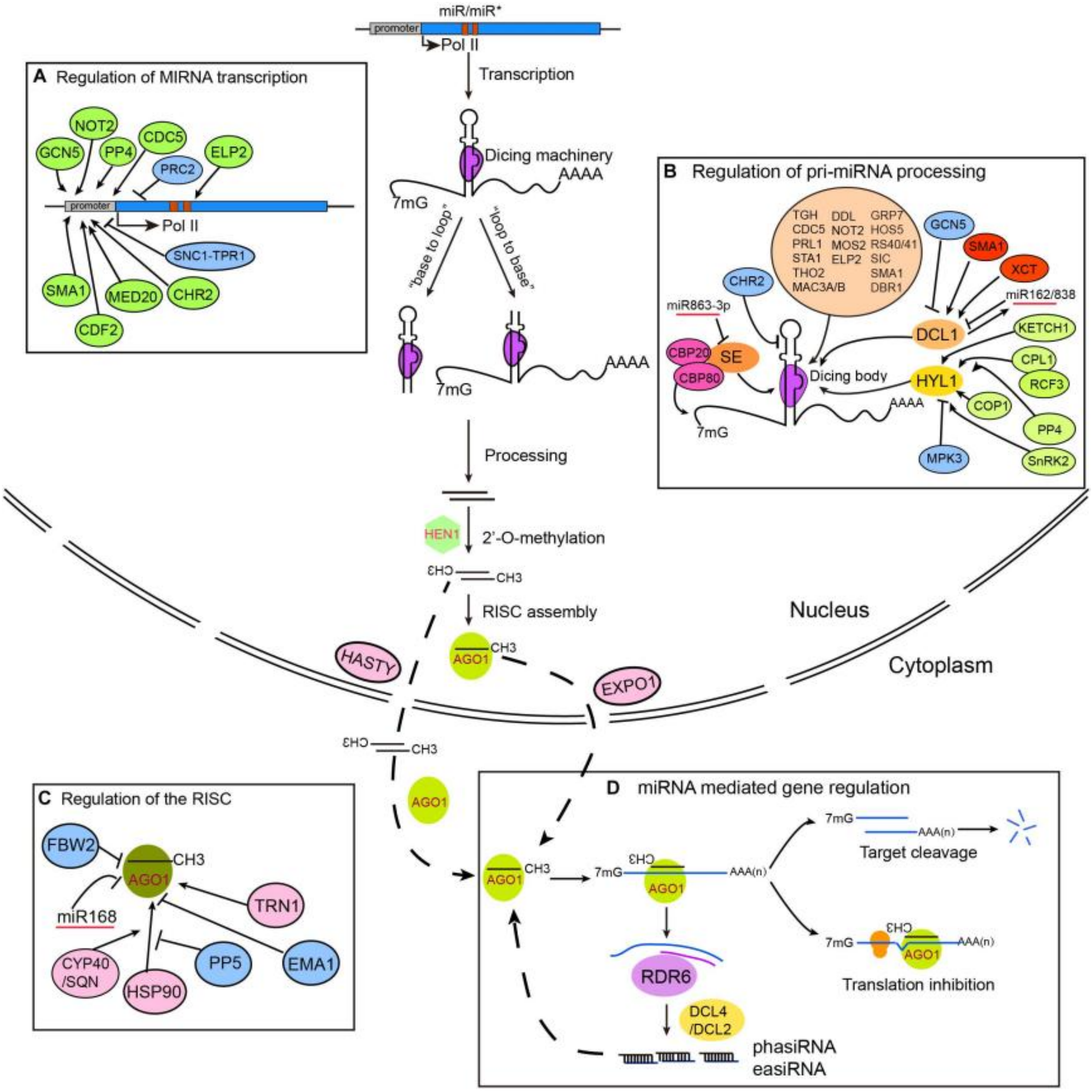

Figure 1 Regulation of miRNA biogenesis, RISC loading, and action in plants. *MIRNA* genes are transcribed by RNA Polymerase II (Pol II) and fold into stem-loop structures called pri-miRNAs. pri-miRNAs are mainly processed by DCL1 from either "base-to-loop" or "loop-to-base" direction. Nascent miRNA/miRNA duplexes are methylated by the small RNA methyltransferase HEN1. RISC loading occurs in the nucleus, but may also occur in the cytoplasm. miRNAs mediate gene silencing through either target cleavage or translation inhibition. Some miRNAs can trigger the production of secondary siRNAs through sequential actions of RDR6 and DCL2/4, generating 21-22 nt secondary siRNAs called phasiRNAs and easiRNAs, which in turn repress gene expression *via* PTGS (for phasiRNAs) or TGS (for easiRNAs) (D). It is important to note that although the steps in the model are separate, they could be closely coupled. Factors involved in the regulation of *MIR* transcription, pri-miRNA processing, and RISC assembly are shown in parts (A-C), respectively (Adopted from Wang et al., 2019)

3.3 Specificity of microRNA-target gene recognition in plants

When it comes to miRNA and target recognition, what usually comes to mind is a "perfect match". The recognition of plant mirnas is indeed very picky. In most cases, only when the bases correspond one-to-one (especially at positions 10 and 11) can cleavage be initiated. The target site may occur in the coding region of mRNA or in the untranslated region. This almost exacting complementarity determines the precision of its regulation. However, there are exceptions - some studies have found that certain mirnas can recognize non-classical binding sites, which has led people to reconsider whether their regulatory scope is broader than previously thought. In addition to sequence complementarity, the structure of the target mRNA itself and the type of AGO protein can also affect the silencing efficiency (Liu et al., 2017; Ajila et al., 2023).

4 Expression Profiling of microRNAs in Rye under Drought Stress

4.1 Application of high-throughput sequencing in miRNA research

To understand the expression status of miRNA in plants under stress conditions such as drought, relying solely on traditional methods is far from sufficient. High-throughput sequencing (HTS) technology, especially next-generation sequencing (NGS), has taken a dominant position in miRNA research precisely because it can rapidly scan and quantify mirnas across the entire genome. This type of technology can not only capture conserved mirnas but also discover new candidate molecules. Typically, the results of HTS are also combined with bioinformatics analysis, such as predicting the interaction relationship between miRNA and its target mRNA, and the subsequent verification work is generally accomplished by means such as qRT-PCR and RACE PCR (Zhakypbek et al., 2025). This "screening first, then verification" process has become a routine operation for studying the mechanism of miRNA's action in plant stress responses.

4.2 Identification of differentially expressed miRNAs induced by drought stress

Not all mirnas respond to drought, but through HTS comparative analysis, a batch of "drought-sensitive" mirnas have indeed been found in many crops. In crops such as rice, wheat, barley and alfalfa, the expression levels of miR156, miR159, miR164, miR169, miR172, miR396 and miR398 have all changed under drought conditions, with some being activated and others suppressed. The specific situation also depends on the variety, tissue type and degree of stress (Ferdous et al., 2017; Qiu et al., 2020). Such expression differences often carry strong tissue specificity or genotype differences, which also indicates that these mirnas may play a relatively detailed role in drought adaptation. For rye, identifying these differentially expressed mirnas is undoubtedly a crucial step in understanding its drought resistance mechanism.

4.3 Screening and functional prediction of key drought-related miRNAs

Identifying the candidate mirnas is just the starting point. The next key point is to figure out which genes they regulate and the roles of these genes in drought resistance. Through target gene prediction and functional enrichment analysis, it can be seen that many mirnas are related to ABA signaling, root development, antioxidant response, metabolic regulation and other links (Sharma et al., 2025). Regulatory pathways such as the effect of miR164 on NAC transcription factors, the regulation of MYB by miR159, and the regulation of NFYA by miR169 have all been widely mentioned in the context of drought stress (Liu et al., 2019; Zhakypbek et al., 2025). Many studies have further conducted transgenic or expression verification. For example, miR408, once overexpressed in ryegrass, not only improved the water state of the leaves, but also enhanced the antioxidant capacity (Hang et al., 2020). Although these results are not yet the end, they have already demonstrated that the value of miRNA as a breeding tool is becoming increasingly clear.

5 Functional Analysis of miRNA Target Genes in Rye

5.1 Target gene prediction and functional annotation

Which targets exactly does miRNA regulate? This matter needs to be judged by some "accurate" tools. Methods such as psRNATarget, TargetFinder, and degradation omics sequencing are widely used in plant research, especially in finding mrnas complementary to miRNA sequences in rye or other cereals (Figure 2) (Tang et al., 2022). However, prediction alone is not enough. The key lies in what exactly these target genes have done. Very often, they encode transcription factors (such as NAC, MYB, SPL, etc.), signaling proteins, or enzymes involved in ABA signaling, ROS detoxification and root development. These functions are basically closely related to drought resistance (Aravind et al., 2017). Like miR156, miR164 and miR159, the corresponding targets are *SPL*, *NAC* and *MYB* genes respectively, and they are basically all "involved" in the stress pathway. Of course, these regulatory relationships cannot be merely guessed. Experimental evidence like qRT-PCR and RACE-PCR has indeed verified the negative regulatory relationship between miRNA and target genes, which can be regarded as solid evidence for these regulatory mechanisms.

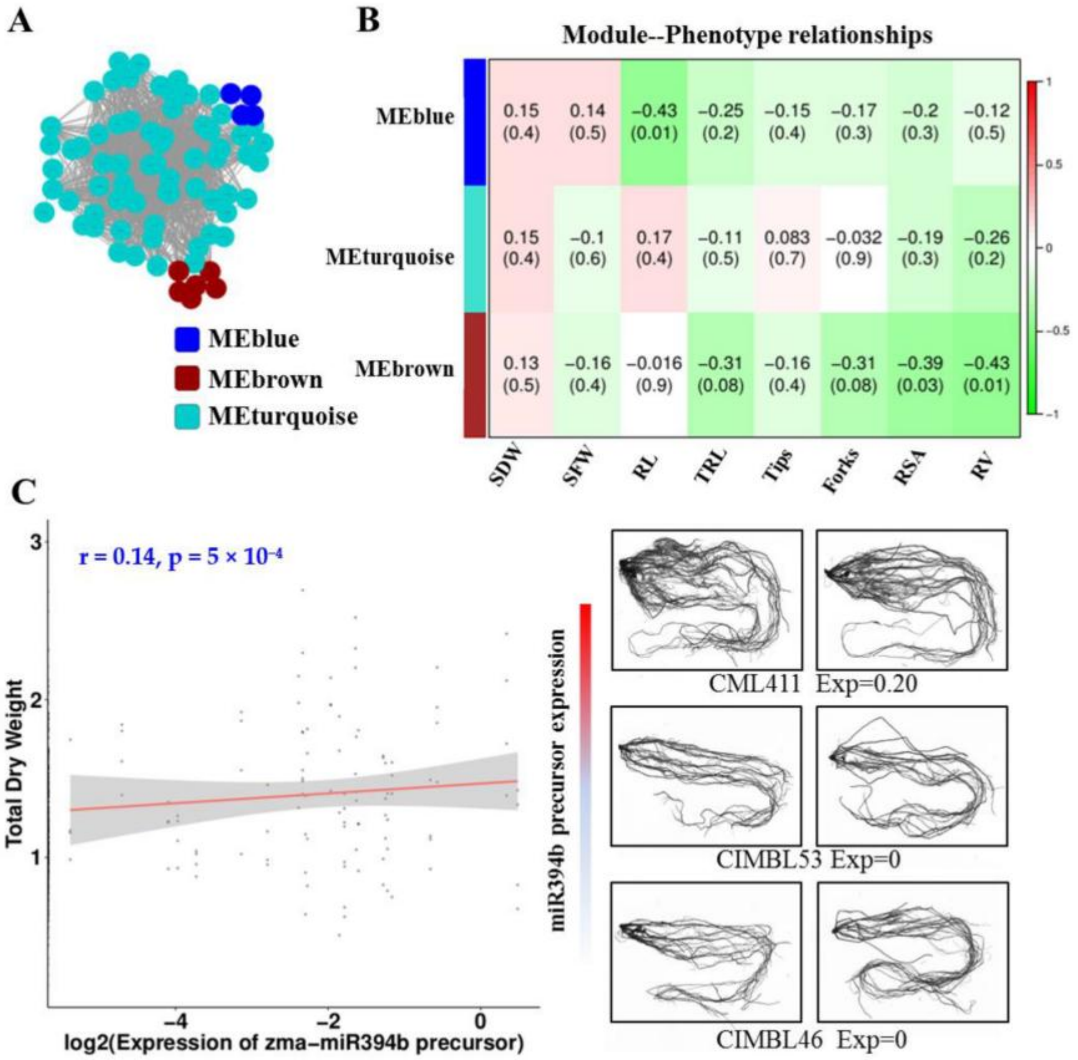

Figure 2 Co-expression network analysis in well water and water stress treatments. (A) Network visualization in Cytoscape. The nodes were colored by module membership. (B) Correlations between module eigengenes and root phenotypic traits. The numbers within the heatmap represent correlations and p-value (red, positively correlated; green, negatively correlated) for the module ‐ trait associations (SDW, shoot dry weight; SFW, shoot fresh weight; RL, root length; TRL, total root length; Tips, root branches; Forks, root forks; RSA, root surface area; RV, root volume). (C) The connection between zma-miR394b precursor expression and total dry weight. On the left is the root phenotype of some lines from a natural group containing 368 lines. Red means that the expression of zma-miR394b precursor is higher (right) (Adopted from Tang et al., 2022)

5.2 Construction of miRNA-target gene regulatory networks

Does one miRNA affect one gene? Sometimes it's really not that simple. Under drought stress, many mirnas and their target genes actually interact in patches and networks. The common practice nowadays is to combine high-throughput sequencing, transcriptome data and co-expression information for analysis, so as to piece together the complete mirNA-target gene network (Sharma et al., 2025). Some "combination punks" are commonly seen in these networks, such as modules like miR156-SPL, miR159-MYB, and miR1119-MYC2, which sometimes carry tissue specificity and genotype differences (Li et al., 2022; Zhakypbek et al., 2025). Methods like WGCNA or degradation groups have also been of great help. They have enabled us to clearly see which mirnas are negatively correlated with the expression of their targets and which ones are in key positions in the drought response.

5.3 Expression patterns and signal integration of target genes in drought response

Not all mirnas under drought stress are "completely suppressed". Some upregulated mirnas push down the target genes involved in growth and signaling pathways, which is a kind of "throttling" response. However, some mirnas themselves were inhibited by drought, and as a result, their target genes could be expressed more. This has become another drought resistance strategy (Shamloo-Dashtpagerdi et al., 2023). This is not just a matter between miRNA and target genes; there are also a bunch of signaling pathways such as ABA and ROS involved, making the entire regulatory system more refined. For example, miR408 in ryegrass, the target genes it controls enhance the drought tolerance of plants by affecting leaf structure and antioxidant function (Hang et al., 2020). Overall, the system composed of miRNA and target genes is like a complete command system for plants to mobilize their drought resistance response. At critical moments, it can also integrate different signal sources for overall coordination.

6 Case Studies: Roles of Key miRNAs in Rye Drought Tolerance

6.1 Functional analysis of miR398 in regulating the ROS scavenging system

When it comes to how plants respond to oxidative stress under drought conditions, miR398 is basically an unavoidable role. It is quite "ancient" and conservative among many plants, but when it really works, it depends on how its expression state changes. Under drought conditions, miR398 is often not elevated but inhibited. As a result, the target genes it originally controlled, such as copper/zinc superoxide dismutase (CSD1 and CSD2), start to be upregulated. Such enzymes happen to be the main force in eliminating ROS and are quite crucial for maintaining the stability of the cellular environment (Li et al., 2022). Of course, this kind of regulation is not the more the better. Instead, it is a dynamic "delegation of power" behavior carried out by the plant itself within its regulatory system, with the aim of "speeding up" the antioxidant system. Many studies, including cases of crops such as grains, have demonstrated a point: regulating miR398 can indeed help plants enhance their "self-protection" ability to some extent when facing drought.

6.2 miR159-mediated regulation of the ABA signaling pathway

Small Rnas like miR159 are also frequently mentioned in the mechanisms by which plants respond to drought. Its activity level will change with the occurrence of drought or the increase of ABA levels. Interestingly, although it is induced by ABA, it in turn acts on some core downstream links of ABA, especially regulating the transcription factors of the MYB family, such as MYB33, and MYB33 in turn affects the expression of key response genes like *ABI5*. It's a bit convoluted, but the logic is clear: drought → increased ABA → increased miR159 expression → suppressed MYB33 → ABI5 regulates adaptive response (Singroha et al., 2021; Jiang et al., 2022). In *Arabidopsis thaliana*, if the activity of miR159 is artificially reduced, the drought resistance of the plants becomes even stronger, indicating that this pathway does have a certain "braking" effect. This regulatory chain has also found similarities in cereal crops. As for whether it operates in the same way in rye, more experiments are needed for further confirmation at present.

6.3 Role of miR166 in leaf development and water retention

As for miR166, its main focus is not on ROS or hormone pathways, but on leaf development. It affects the morphology and structure of leaves by regulating the expression of HD-ZIP Class III transcription factors, and thereby indirectly influences the plant's ability to retain water. Studies have found that after reducing the activity of miR166, the leaf morphology of plants changed and the transpiration rate slowed down, all of which were related to the improvement of drought tolerance (Singh et al., 2022). HD-ZIP III is not only related to vascular development but also to leaf polarity. Therefore, the regulatory range of miR166 is actually quite broad. At present, this miRNA has considerable potential in regulating the water use efficiency of plants under drought conditions, especially in crops like rye that have high requirements for environmental adaptation, which is even more worthy of in-depth research (Lang et al., 2025).

7 Conclusions and Future Perspectives

When it comes to how plants survive drought, the role of miRNA cannot be bypassed. There have been numerous studies in the past, and it is widely recognized that miRNA plays a crucial role in regulating plant drought resistance. For instance, it can influence abscisic acid (ABA) signaling, eliminate reactive oxygen species (ROS), and shape root structure, among other important processes. Drought-responsive mirnas such as miR156, miR159, miR164, miR172 and miR398 usually target transcription factors and some stress-related genes. A complete set of complex regulatory networks (miR156, miR159, miR164, miR172 and miR398) has been constructed. The identification of these mechanisms is attributed to high-throughput sequencing and integrated bioinformatics tools, which enable us to more systematically identify the existence and targets of these mirnas, thereby advancing our understanding of post-transcriptional regulation in cereal crops and laying the foundation for breeding applications.

However, the research has not been smooth sailing all the way, especially when it comes to truly applying these achievements to breeding, there are still many obstacles to overcome. For instance, under different genotypes and experimental conditions, the expression patterns of the same miRNA often vary, and the tissue specificity is also obvious, which makes the induction of "universal mirnas for drought response" quite challenging. Furthermore, although targets can now be predicted through tools like psRNATarget and TargetFinder, and verified by means such as RACE-PCR, qRT-PCR, and degradation omics sequencing, the entire process has become relatively mature. However, the operational costs and data processing pressure remain considerable. One more point that is often overlooked is that species like rye, which are valuable in agriculture but have received little research, also urgently need to be followed up. We should not always focus on a few model plants. New technologies such as single-cell sequencing and AI prediction may be breakthroughs to enhance the accuracy of miRNA annotation and functional recognition, but at present, they are still in their infancy.

By the way, the functional research of miRNA has still opened up many new paths for crop improvement. Mirna-based methods such as overexpression, knockdown, and CRISPR/Cas9 editing have already achieved "good data" on some model plants and major food crops. Whether similar effects can be achieved on rye in the future depends on the specific implementation. Judging from the current trend, whoever can safely incorporate the experimental results into the breeding system will be able to add an extra layer of security to food security in the context of increasingly frequent droughts.

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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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