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AN UPDATE ON MICRORNAS: EMERGING ROLES IN PLANT GROWTH, DEVELOPMENT, AND STRESS ADAPTATION

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ABSTRACT

As key threats to global crop productivity, environmental stresses trigger sophisticated multi-level regulatory mechanisms in plants, spanning transcriptional to translational control. Among these, microRNAs (miRNAs) have emerged as pivotal post-transcriptional regulators. This review details miRNA biogenesis, function, and research methodologies, focusing on their dynamic roles in abiotic stress responses, including temperature extremes, drought, salinity, and heavy metals. By differentially expressing under stress, miRNAs fine-tune gene networks through repression of negative regulators and enhancement of positive responses. Furthermore, we synthesize their broader roles in plant metabolism and development, offering a comprehensive overview of miRNA discovery, function, and evolution to advance understanding of plant stress adaptation.

KEYWORDS: microRNAs; Abiotic Stress; Gene Regulation; Plant Development; Crop Productivity

1. INTRODUCTION

MicroRNAs (miRNAs), characterized as endogenous non-coding single-stranded small RNAs (approximately 18–24 bp), are ubiquitous across animals (Ambros, 2004), plants (Zhan et al., 2023), and microorganisms (Pinhal et al., 2024). Science their initial characterization in nematodes in 1993 (Lee et al., 1993), the field has been profoundly advanced by techniques like high-throughput sequencing and bioinformatics (Wang et al., 2020; Kuang, et al., 2023). This has enabled the discovery of a vast repertoire of miRNAs, both conserved and species-specific, in plants ranging from *Arabidopsis thaliana* (Lu et al., 2017; Tiwari et al., 2020; Padilla-Padilla et al., 2024), and moss to crucial crops like rice (Zhou et al., 2024), maize (Chen et al., 2020; Chen et al., 2024), wheat (Lei et al., 2021; Qiao et al., 2023), underscoring their pivotal regulatory functions in diverse biological pathways. Throughout the life cycle, plants are inevitably exposed to a multitude of environmental stresses, encompassing both biotic threats (e.g., pathogens, pests) and abiotic adversities (e.g., extreme temperatures, drought, salinity, nutrient deficiency) (Mareri et al., 2022; Wang et al., 2024). These stresses can severely impair physiological processes, inhibit growth and development, and ultimately lead to substantial losses in crop yield and quality (Ding and Yang, 2022; Nichol et al., 2023; Zhou et al., 2024). To counter these challenges, plants have evolved sophisticated response mechanisms, with miRNA-mediated post-transcriptional gene regulation playing a central, orchestrating role (Pu et al., 2019; Zhou et al., 2024; Niu et al., 2025). The specific expression profiles of miRNAs dynamically shift in response to the type and severity of stress. These changes function as precise "molecular switches", reprogramming the plant's physiology by modulating target gene expression, either upregulating defense pathways or downregulating negative regulators, thereby enhancing stress tolerance and ensuring survival (Premachandran, 2022). Consequently, miRNAs are indispensable for plant stress perception and adaptive response. The mechanisms of action of plant and animal miRNAs differ significantly, reflecting their distinct evolutionary trajectories (Song et al., 2019; Kim et al., 2025). A key distinction lies in their target recognition and silencing strategies. Plant miRNAs typically exhibit near-perfect complementarity to the coding regions of their target mRNAs. This high degree of specificity enables them to function as "molecular scissors," guiding Argonaute (AGO) proteins to mediate endonucleolytic cleavage and subsequent degradation of the transcript, resulting in precise gene silencing (Liang et al., 2023). In contrast, animal miRNAs generally bind with imperfect complementarity to sites within the 3' untranslated regions (3' UTRs) of their targets, primarily leading to translational repression (Bartel, 2009). These functional differences are underpinned by divergent biogenesis pathways. Plant miRNAs originate from independent MIR genes. Their primary transcripts (pri-miRNAs) undergo precise, stepwise processing in the nucleus by the Dicer-like1 (DCL1) enzyme complex, which is aided by co-factors like HYPONASTIC LEAVES1 (HYL1) and SERRATE (SE). This process generates stem-loop-structured

precursor miRNAs (pre-miRNAs) and culminates in the production of mature miRNA duplexes. These duplexes are then stabilized through 3' end methylation by HUA ENHANCER1 (HEN1) and exported to the cytoplasm via HASTY (HST). Finally, the guide strand of the mature miRNA is incorporated into an Argonaute (AGO) protein to form the RNA-induced silencing complex (RISC), which seeks out and silences complementary target mRNAs (Moro et al., 2019).

Plant miRNAs are now recognized as master regulators, with confirmed roles in a wide array of developmental processes, from organogenesis to floral transition, and in adaptive responses to abiotic stresses like extreme temperatures, drought, and salinity (Owusu Adjei et al., 2021; Raza et al., 2023). For instance, miR408 controls stomatal movement, thereby coordinating photosynthetic growth with drought tolerance (Yang et al., 2024). In panax ginseng, the interaction between miRNA156 and the PgSPL24-09 gene regulates the growth of adventitious roots (Jiang et al., 2024). Even modest, stress-induced alterations in miRNA levels can initiate cascading effects through signaling networks by modulating critical targets, including transcription factors (Feng et al., 2023). This results in a wholesale restructuring of cellular metabolism and defense mechanisms, thereby boosting overall resilience. Therefore, deciphering the intricacies of miRNA biogenesis, action, and their integrated networks is pivotal. This knowledge is dual-purpose: it systematically unravels the complex, multi-tiered regulatory networks governing gene expression in plants, and it directly informs modern breeding paradigms (Sasi et al., 2023;). The targeted manipulation of key MIR loci via gene editing, or the fine-tuning of miRNA activity using technologies like MIMICs/STTMs, paves the way for engineering a new generation of crops endowed with superior, broad-spectrum stress resistance (Rabuma et al., 2025). This molecular toolbox is crucial for devising innovative strategies to safeguard food production in an era of global climate change.

2 Research on miRNA in Plant Growth and Development

2.1 The Role of miRNA in Plant Root Development

Plant roots are essential organs responsible for water and nutrient uptake, plant anchorage, and environmental interactions (Fan et al., 2025). Their development is governed by a complex and precise gene regulatory network. miRNAs, a class of endogenous non-coding small RNAs, function as key “fine-tuners” and “signaling hubs” in root development, modulating multiple layers of this process through post-transcriptional silencing of target genes (Barrera-Rojas et al., 2021). Jiang et al. demonstrated that miRNA156 interacts with the age-related gene *PgSPL24-09* and represses its expression. Their study elucidates the molecular mechanism underlying the regulation of ginseng adventitious root growth by the miRNA156-SPL module (Jiang et al., 2024). Overexpression of sly-miR156 leads to the development of dense aerial roots on the stems of tomatoes and tobacco (Zhang et al., 2011). Xu et al. (2017) reported that treatment with indole-3-butyric acid (IBA) resulted in a significantly higher adventitious rooting rate in juvenile cuttings than in mature cuttings of apple rootstock *Malus xiaojinensis*. Furthermore, expression analysis revealed that mxi-miR156 levels were substantially elevated in juvenile cuttings. Thus, researchers hypothesized that high mxi-miR156 expression is essential for adventitious root formation in this species. The subsequent experimental validation demonstrated that mxi-miR156 promotes rooting by downregulating *MxSPL26*, and concurrently upregulating key rooting-related genes like MxRTCS-like (Xu et al., 2017). In *Arabidopsis* root, ARGONAUTE10 regulated root meristem activity and xylem patterning by degrading miR165/166 to modulate PHB expression gradients (Mirlohi et al., 2024). The *crd1* mutation in rice, disrupting the *HASTY* ortholog *CRD1*, impairs miRNA export and reduces miR156 activity, thereby inhibiting crown root development.(Zhu et al., 2019). The small peptide vvi-miPEP171d1 specifically promoted adventitious root formation in grapevine by activating its cognate pri-miRNA expression, demonstrating species-specific regulation of root development by miPEPs (Chen et al., 2020). Huang et al. (2019) further demonstrated that in citrus, csi-miR171 and csi-miR319 regulate root development by targeting MYB (MYELOBLASTOSIS) and SCARECROW family genes, respectively. This regulatory mechanism enables normal root elongation under boron toxicity, playing a crucial role in conferring boron tolerance in citrus (Huang et al., 2019). During turnip storage root development, brp-miR156a, brp-miR157a, and brp-miR172a exhibit markedly elevated expression levels. These miRNAs demonstrate distinct expression patterns during both root formation and secondary thickening phases, and their expression profiles are negatively correlated with those of their corresponding target genes, underscoring the significant regulatory role of miRNAs in turnip storage root development (Li et al., 2015).

2.2 The role of miRNA in Plant Leaf Development

The leaf, a vital vegetative organ in plants, drives growth and development by synthesizing organic compounds through photosynthesis. The initiation of this organ is primarily regulated by the distribution and concentration gradient of the hormone auxin (Lu et al., 2025). Research has demonstrated that microRNAs (miRNAs) play a significant role in leaf development of horticultural crops, mainly through their involvement in auxin efflux pathways to regulate leaf morphogenesis (Li et al., 2016; Sang et al., 2023). ARFs (Auxin Response Factors) are transcription factors involved in auxin signaling during many stages of plant growth and development (Liu et a., 2007). Through the application of STTM technology, Damodharan et al. downregulated sly-MIR160 in tomato, leading to upregulated expression of its target genes sly-ARF10A and sly-ARF17, which subsequently suppressed leaf growth and resulted in a significant reduction in leaf area. This demonstrates the critical regulatory role of

sly-miR160 in tomato leaf development (Damodharan et al., 2016). Liu et al. (2014) conducted a comparative analysis of miRNA expression profiles across sweet orange leaves, flowers, and fruits, revealing that 60 known miRNAs, including csi-miR160, along with 11 novel miRNAs exhibited significantly higher expression in leaves compared to floral or fruit tissues. This observation suggests a potentially important functional role for these miRNAs in leaf biology. Collectively, these findings highlight that while miR160 consistently targets ARF family genes across diverse crop species, its specific regulatory functions may vary in a context-dependent manner (Liu et al., 2014). The morphological development of leaf margins in both simple and compound leaves is regulated by the interaction between auxin and CUP-SHAPED COTYLEDON (CUC) transcription factors. During this process, miR164 modulates leaf margin morphology through the post-transcriptional repression of its target gene CUC2 (Hasson et al., 2011). Song et al. demonstrated that the *Arabidopsis* miR394-LCR module finely tunes leaf development by modulating auxin-responsive pathways, establishing an optimal expression threshold critical for normal leaf morphology (Song et al., 2012). In a diploid woodland strawberry mutant exhibiting deeply serrated leaves, Zheng et al. (2019a) identified a missense mutation at the 19th nucleotide of the fve-miR164 precursor gene. Overexpression of FveMIR164A in this mutant rescued the serrated phenotype, confirming that fve-miR164 regulates strawberry leaf shape development by targeting FveCUC2a (Zheng et al., 2019a). Through eGWAS analysis, Sakuraba et al. identified that HASTY (HST), an importin/exportin protein, orchestrates nitrogen deficiency responses in *Arabidopsis* by modulating miRNA dynamics and their interactions with NAC transcription factors, thereby forming a central regulatory node in the nitrogen deficiency response network (Sakuraba et al., 2024).

miR156 regulated leaf development primarily through its targeting of SPL transcription factors. For example, in passion fruit, heteromorphic leaf formation was associated with decreased ped-miR156 and increased ped-miR172 expression, with further evidence showing that ped-miR156 fine-tunes mature leaf traits by regulating PeSPL9 during early bud development (Silva et al., 2019b). Similarly, in tomato, overexpression of sly-miR156 lead to a phenotype of smaller and more numerous leaves (Zhang et al., 2011). Additionally, sly-miR171 contributed to compound leaf morphogenesis by targeting HAM genes, thereby maintaining shoot and inflorescence meristem stability (Hendelman et al., 2016). miR319 regulated leaf shape by targeting TCP (TEOSINTE BRANCHED 1/CYCLOIDEA/PCF) transcription factors, thereby influencing leaf cell division and differentiation. In the tomato Lanceolate (LA) mutant, large compound leaves were transformed into small simple leaves. LA encoded a transcription factor from the TCP family that contains a target site for sly-miR319. In tomato plants overexpressing sly-miR319, differentiation at the leaf margin was delayed, manifesting as later trichome development and prolonged leaflet differentiation, indicating that sly-miR319 and LA jointly regulate tomato leaf morphology (Ori et al., 2007). In *Arabidopsis*, miR396 is highly expressed during leaf and seedling development (Liu et al., 2009a). Its overexpression was shown to limit cell proliferation, producing a phenotype characterized by narrower leaves, reduced stomatal density, and increased drought tolerance. Subsequently, Rodriguez et al. (2010) demonstrated that miR396 achieves this by regulating the expression levels of key transcription factors, thereby controlling the balance between cell proliferation and differentiation, as well as meristem size (Rodriguez et al., 2010). miR319 also played a significant role in leaf shape development in cruciferous vegetables. Overexpression of Chinese cabbage Brp-MIR319a reduced the expression level of its target gene BrpTCP4 and shifts the shape of the cabbage head from round to cylindrical, demonstrating that brp-miR319a influences head shape by targeting and suppressing BrpTCP4 expression to control leaf cell division (Mao et al., 2014). Thus, the overall role of miR319 in leaf development was consistent in both tomato and Chinese cabbage, promoting cell division and delaying differentiation. Additionally, overexpression of another miRNA precursor gene in Chinese cabbage, Brp-MIR166g-1, altered the direction and extent of leaf curvature during the rosette stage, causing rosette leaves to bend downward instead of lying flat, while folded leaves become flat rather than upward-curving, resulting in reduced leaf curvature and smaller head size (Ren et al., 2018).

2.3 The Role of miRNAs in Plant Flowering and Floral Organ Development

2.3.1 miRNA-Mediated Regulation of FloweringTime

miR156 plays a central role in regulating phase transitions during plant development. The *SQUAMOSA PROMOTER BINDING PROTEIN-LIKE* (*SPL*) family of transcription factors, initially identified in *Antirrhinum majus* for their ability to bind the promoter of the floral meristem-specific gene *SQUAMOSA* (Klein et al., 1996; Schwab et al., 2005), are key targets of miR156. By directly repressing SPL expression, miR156 controls the shift from vegetative growth to reproductive development (Wang, 2024). miR156 expression is maintained throughout juvenile vegetative stages, where it functions as a critical molecular determinant of the juvenile-to-adult phase change. Its levels gradually decline as plants mature. The miR156-targeted SPL genes, in turn, regulate a subset of MADS-box genes, thereby governing the subsequent transition from the adult to the reproductive phase (Wang et al., 2009). A dynamic miR156-SPL module orchestrates flowering timing. Declining miR156 abundance permits the gradual accumulation of SPL transcripts. Once a threshold of SPL expression is attained, downstream floral pathway genes are activated, leading to flowering initiation. Studies indicate that miR156 constitutes an endogenous flowering pathway (Zhou and Yarra, 2023), modulation of its levels provides a genetic mechanism that enables plants to flower autonomously, even without external environmental cues.

miR172, like miR156, acts through mRNA degradation and translational repression to modulate flowering time and floral organ formation (Su et al., 2023). Li et al. demonstrated that manipulating miR172 expression effectively controls flowering time in gloxinia by targeting SsAP2-like, offering a universal strategy for regulating flowering in ornamental plants (Li et al., 2019). Rao et al. revealed that autotetraploid *Lycium ruthenicum* exhibits delayed flowering due to polyploidy-induced modulation of the miR156-SPL and miR172-AP2 age pathways, which suppressed FT expression and subsequently inhibit *SOC1* in the shoot apical meristem (Rao et al., 2021). The conserved miR172 promoted flowering in wheat by repressing APETALA2-like genes, which fine-tuned the flowering response by regulating FT1 independently of the core vernalization pathway and interacted with age-related and vernalization signals (Debernardi et al., 2022). Studies in the short-day plant *Petunia hybrida* (Glazinska et al., 2009) further illustrate this regulatory relationship: under long-day conditions, elevated expression of AP2-like genes coincides with reduced miR172 levels. Conversely, auxin or ethylene treatment, as well as the elimination of a night interruption during photoperiodic induction, leads to decreased AP2-like expression and a corresponding increase in miR172 accumulation. Moreover, Grigorova et al. showed that mutations in the miR172-binding site of AP2-like genes cause severe defects in *Arabidopsis* floral organ development, while downregulation of AP2-like genes is associated with miR172 expression in specific floral whorls (Grigorova et al., 2011). Collectively, these findings underscore the essential, antagonistic roles of miR172 and AP2-like genes in flowering-time control. These findings collectively confirm the crucial roles of miR172 and AP2-like genes in flowering time regulation. Furthermore, within the miRNA-ediated control of the plant growth cycle, miR156 and miR172 function in a coordinated manner: miR156 represses the expression of SPL family genes, whereas specific SPL proteins subsequently enhance miR172 expression.

Both miR159 and miR319 are functional microRNA families that play important roles in regulating floral development in plants. Overexpression of either miRNA leads to abnormal flower development, such as delayed flowering (Hu et al., 2019), highlighting their key regulatory functions in plant development. The target genes of miR159 and miR319 are the MYB and TCP transcription factor families, respectively. Sequence homology analysis shows a high degree of similarity in their nucleotide composition, but the two miRNAs do not cross-regulate each other. Research has demonstrated that overexpression of miR159 delays flowering under short-day conditions (Li et al., 2013), whereas overexpression of miR319 delays flowering under long-day conditions (Silva et al., 2019), suggesting different regulatory mechanisms for these two miRNAs. Additionally, gibberellin has been shown to enhance the expression of miR159.

2.3.2 Regulation of Floral Organ Development by microRNAs

Beyond their established roles in flowering time regulation, miR156 and miR172 are critically involved in floral organ development across various plant species. Heterologous expression of apple mdm-miR156h in *Arabidopsis* not only prolonged juvenility and increased leaf number but also induced floral organ abnormalities, alongside partial silique and seed abortion (Sun et al., 2013). In chestnut, cmo-miR156 shows preferential expression in male flower clusters and stamens, with levels approximately double those in female flowers (Chen et al., 2019). Similarly, in Korla fragrant pear, members of the psi-miR156 family exhibit differential expression in sepal tissues, with higher levels associated with sepal abscission, suggesting a potential regulatory role in this process (Ma et al., 2020). Overexpression of sly-miR172 in tomato results in enlarged sepals, narrowed petals, and homeotic sepal-to-petal conversions (Chung et al., 2020). Conversely, loss-of-function mutations in sly-miR172c and sly-miR172d lead to distinct phenotypes: petals and stamens turning slightly green, or transforming into sepalloid organs, respectively (Lin et al., 2021). In peach, disruption of the ppe-miR172 binding site on an AP2 transcription factor causes an increase in petal and stamen number (Gattolin et al., 2018). Kiwifruit studies further confirm that ach-miR172 targets AP2, and its loss leads to aberrant flower development (Varkonyi-Gasic et al., 2012).

Other miRNAs also contribute to floral organ specification. Silencing sly-miR160 in tomato yields narrow sepals and petals and delays petal/anther abscission, a phenotype linked to the upregulation of *SlARF10A* and *SlARF17* (Damodharan et al., 2016). fve-miR164a mutants in diploid wild strawberry exhibit serrated petal/sepal margins, abnormal carpels, and sterility due to *FveCUC2a* overexpression (Zheng et al., 2019a). In Gloxinia (*Sinningia speciosa*), suppressing ssp-miR159 via STTM leads to partial sepal-to-petal conversion, disrupting normal sepal identity (Li et al., 2013).

2.4 Regulation of Pollen Development by miRNA

As a critical component of floral development, pollen development significantly influences the breeding, as well as the seed yield and quality, of horticultural crops (Foubert-Mendes et al., 2025). Accumulating evidence has demonstrated that microRNAs (miRNAs) play pivotal regulatory roles in pollen development among cruciferous plants. In Chinese cabbage, Ma et al. (2017) identified and cloned two precursors of bra-miR158 (bra-MIR158a1 and bra-MIR158a2) along with its target gene bra027656. These genes exhibited high expression levels in inflorescences, and overexpression of bra-miR158 was found to significantly reduce the pollen germination rate (Ma et al., 2017). Comparative analysis by Jiang et al. (2014) of miRNA expression in flower buds between a male-sterile line (Bcajh97-01A) and its maintainer fertile line (Bcajh97-01B) of Chinese cabbage revealed 18 miRNAs with significantly differential expression (Jiang et al., 2014). Furthermore, Li et al. (2017) reported consistently high expression of specific miRNAs, including bol-miR167, bol-miR166, bol-miR156/157, bol-

miR165, bol-miR158, and bol-miR168, across three early developmental stages of cauliflower pollen, underscoring their active involvement in microspore development (Li et al., 2017). Collectively, these findings strongly implicate miRNAs in regulating pollen fertility and developmental processes in cruciferous vegetables.

2.5 The Role of miRNA in Fruit Development

2.5.1 Regulation of Fruit Development by miRNA

miRNAs implicated in fruit development have been identified across a range of horticultural crops, including tomato, pepper, strawberry, sweet orange, and peach. The miR160 family represents another significant class of miRNAs involved in regulating fruit development. Research has demonstrated that overexpression of tomato sly-miR160a in transgenic plants not only causes narrowing of leaves, sepals, and petals but also leads to the formation of fruits with abnormal morphology (Hendelman et al., 2012). Conversely, suppression of sly-miR160 expression results in aberrant ovary development, pear-shaped fruits, and the inhibition of fruit abscission in tomato (Damodharan et al., 2016). Further investigation revealed that the molecular basis of this phenotype involves sly-miR160a modulating the expression of SlARF10A/10B/17, thereby influencing early fruit development in tomato. Through small RNA high-throughput sequencing, Liu et al. (2017) identified 59 known and 310 novel miRNAs associated with pepper fruit ripening. Subsequent qRT-PCR analysis confirmed that three of these miRNAs (can-miR156a, can-miR160a, and can-miR396a-5p) are highly expressed in fruits at 50 days post-anthesis, coinciding with the downregulated expression of their corresponding target genes.

The miRNA families miR156 and miR172 constitute another important regulatory layer in the fruit development of horticultural crops. For instance, in cucumber, csa-miR156a modulates fruit expansion by targeting the *CsSPL* gene (Sun et al., 2019). Conversely, overexpression of sly-miR156 in tomato leads to a reduction in fruit number, size, and weight (Zhang et al., 2011). Similarly, in apple, lower expression levels of mdm-miR172 correlate with larger fruit size in breeding populations, while its overexpression in transgenic plants results in significantly smaller fruits, confirming its role in determining fruit size (Yao et al., 2015). Beyond miR156 and miR172, additional miRNAs participate in fruit development regulation. In peach, the expression pattern of ppe-miR166 is inversely related to those of its four target genes (PpHB14, PpHB15, PpHB8, and PpREV), suggesting its negative regulatory role throughout fruit development (Zhang et al., 2015). Furthermore, degradome analysis in strawberry identified a novel miRNA, Fa_novel6, which targets the *HERK1* gene, a receptor kinase involved in cell wall modification and elongation, indicating its potential influence on fruit expansion via cell size regulation (Li et al., 2019a).

2.5.2 Regulation of Fruit Ripening and Senescence by miRNA

Fruit ripening encompasses key sensory changes in texture, aroma, and color, representing a critical phase for the development of edible quality. High-throughput sequencing has identified miRNAs involved in regulating fruit ripening and senescence across various horticultural crops, including tomato, papaya, nectarine, blueberry, kiwifruit, strawberry, melon, and pear. Research on strawberry fruit, in particular, has been extensive. For instance, postharvest storage at 4 °C for 0-48 hours delays strawberry fruit senescence. Accompanying high-throughput sequencing revealed a marked upregulation of fan-miR164e and fan-miR164d, with concurrent downregulation of their target NAC transcription factor genes, suggesting fan-miR164 modulates senescence through NAC regulation (Li et al., 2017). Similarly, exogenous ABA treatment reduces fan-miR73 expression, which shows a strong negative correlation with *ABI5* (ABSCISIC ACID-INSENSITIVE 5) transcript levels, indicating that fan-miR73 regulates ripening by targeting ABI5 (Li et al., 2016). Overexpression of fve-miR399a in diploid woodland strawberry significantly enhances fruit quality, including elevated levels of fructose, glucose, soluble solids, and 33 other compounds, demonstrating its role in quality improvement (Wang et al., 2017). Integrated transcriptome and miRNA profiling of strawberry fruit treated with ABA or nordihydroguaiaretic acid (NDGA, a biosynthesis inhibitor) identified 20 known and 6 novel differentially expressed miRNAs as potential key regulators of ripening, likely mediating ABA's effects on hormone balance, pigment biosynthesis, and cell wall degradation (Li et al., 2019).

Beyond strawberry, miRNAs regulate ripening in other fruit crops. In tomato, sly-miR172a overexpression increases ethylene production 3-4 fold from the ripening stage onward and upregulates CYC-B (lycopene β-cyclase), enhancing lycopene-to-β-carotene conversion. This shows sly-miR172 promotes ripening by modulating ethylene biosynthesis and carotenoid accumulation, partly through repression of SlAP2a (Chung, 2020). In melon, cme-miR393-overexpressing lines exhibit delayed ripening, with cme-miR393 acting by suppressing its target CmAFB2 (Bai et al., 2020). Multi-omics analysis of pear under different postharvest temperatures identified 16 temperature-responsive microRNAs interacting with senescence-related mRNAs. Transient expression assays confirmed that Novel_188 accelerates senescence by inhibiting Pbr027651.1 (Gu et al., 2020). Additionally, aar-miR858 shows higher expression in green-fleshed than red-fleshed kiwifruit and regulates anthocyanin composition by targeting AaMYBC1 to control AaLDOX expression, thereby influencing flesh color (Li et al., 2019). Genome-wide analysis in sweet orange identified 183 known and 38 novel miRNAs, with csi-miRN31, csi-miR477a-3p, and csi-miR164a highly expressed in fruit, highlighting their potential as ripening regulators (Liu et al., 2014).

2.6 The Role of miRNAs in the Development of Other Organs

Within the grapevine vvi-miR160 family, vvi-miR160c/d/e and their target gene VvARF18 are implicated in seed development and formation, whereas vvi-miR160a/b and their target gene VvARF18 primarily regulate the gibberellin-induced seed abortion process (Bai et al., 2020). During potato tuber development, both conserved miRNAs (stu-miR172_1 and stu-miR172_5) and specific miRNAs (stu-miR193 and stu-miR152) are significantly upregulated, indicating their likely involvement in tuber formation (Lakhotia et al., 2014). Studies further demonstrate that in potato, miR172 overexpression or silencing of PHYB (PHOTORECEPTOR PHYTOCHROME B) downregulates the expression of its target gene APETALA2-like. Additionally, miR172 overexpression upregulates *BEL5*, suggesting that the stu-miR172-APETALA2 regulatory module operates downstream of the tuberization suppressor PHYB and upstream of the tuberization promoter *BEL5* (Martin et al., 2009). Beyond stu-miR172, stu-miR156 also participates in tuber development by regulating *StSPL9* (Bhogale et al., 2014). Under short-day conditions, stu-miR156 expression increased 2.5-fold in shoot tips and two-fold in stolons compared to long-day conditions. Overexpression of stu-miR156 in potato alters plant architecture and reduces tuber yield (Kumar et al., 2020). In Dendrobium catenatum (syn. D. officinale) seedlings, dca-miR156 accumulates abundantly in protocorms during early developmental stages and declines as seedlings mature. Concurrently, its target genes *DcSPL14*, *DcSPL7*, and *DcSPL18* show increased transcription levels, indicating that dca-miR156 plays a critical role in protocorm development (Zheng et al., 2019b). Furthermore, csi-miR156 is involved in citrus somatic embryogenesis. Overexpression of csi-miR156a or knockout of its target genes *CsSPL3* and *CsSPL14* significantly enhances somatic embryogenesis capacity in citrus callus, highlighting the regulatory role of the csi-miR156-SPL module in the induction and establishment of citrus somatic embryos (Long et al., 2018).

3 miRNA and Stress Response

During growth and development, plants experience varying levels of both biotic and abiotic stress, such as temperature extremes (González-García et al., 2023), water scarcity (Bagal et al., 2025), high salinity (Segarra-Medina et al., 2025), and pest or pathogen attacks (Kumari et al., 2024). Under such adverse conditions, plant miRNAs regulate target gene expression by binding to complementary mRNAs, which inhibits translation or triggers mRNA degradation. This post-transcriptional regulatory mechanism enables plants to adapt to and mitigate the effects of environmental challenges (Song et al., 2019).

3.1 miRNAs and Temperature Stress

Temperature is one of the most common and influential factors affecting plant growth and development. Both excessively high and low temperatures can impair growth and reduce crop yield and quality. Low-temperature stress disrupts photosynthesis and hinders normal development. In 2004, miRNAs involved in low-temperature stress were first identified, and their expression profiles were analyzed using microarrays. Subsequent studies revealed that miR393, miR396, and miR397 exhibited expression changes of up to 1.5-fold under low-temperature stress in various plant species, including Arabidopsis, rice, and poplar. Among them, miR393 is induced under low-temperature and high-water conditions, where it suppresses the expression of its target genes TIR1 and AFB2, thereby enhancing plant adaptability to stress (Sunkar and Zhu, 2004). Under low-temperature stress, Arabidopsis miR396 regulates its target GRF genes, which interact with DELLA proteins, key components of cold-induced auxin signaling, to promote growth (Guo et al., 2016). In trifoliate orange, prolonged low-temperature stress leads to a gradual increase in miR396 expression, while the expression of its target gene ACO decreases, showing a significant negative correlation (Ouranin et al., 2020). In rice, high-temperature stress induces the expression of OsCSD2 while significantly reducing miR398a expression, indicating a negative correlation. Further analysis showed that miR398a cleaves its target OsCSD2, mitigating the negative effects of high-temperature stress. Similarly, in tomato, the expression of SlymiR171d increases with prolonged high-temperature treatment, accompanied by a decrease in its target gene SCL6, contributing to thermotolerance (Zhou et al., 2020).

3.2 miRNAs and Water Stress

Soil water deficiency is a primary cause of drought conditions. Prolonged drought impairs nutrient uptake, causes leaf wilting, and may damage reproductive tissues, thereby disrupting normal plant growth and development. Under drought stress, certain miRNAs help plants survive by regulating various aspects of growth and development. In Arabidopsis, miR172e, which is regulated by the photoperiod factor GI, is induced under drought stress, suppressing its target genes and promoting early flowering as an escape strategy (Han et al., 2013). In alfalfa, miR156 modulates physiological and biochemical traits such as stomatal conductance and hormone balance in leaves and roots under drought, contributing to stress adaptation (Arshad et al., 2017).

3.3 miRNAs and Salt Stress

Plant responses to salt stress are dynamic and concentration-dependent. As salt concentration increases, plant tolerance initially rises but subsequently declines; beyond a critical threshold, normal growth and development are inhibited. Multiple miRNAs, including miR396, miR399f, miR167, and miR160, have been implicated in salt

stress responses (Yuan et al., 2024). In birch, BpmiR408 responds to salt stress by suppressing the expression of its target gene BpCBP1 (Wang et al., 2020). Qiao et al. (2023) profiled miRNA expression in bread wheat under salt stress using high-throughput sequencing. A total of 360 conserved and 859 novel miRNAs were identified, with 49 differentially expressed, of which 25 were upregulated and 24 downregulated. qRT-PCR validation confirmed the sequencing trends, notably higher expression of miR109 and lower expression of miR60 and miR202 under salt stress. Among differentially expressed miRNAs, 21 were selected for target prediction, yielding 1,023 candidate targets. GO and KEGG analyses revealed involvement in processes such as RNA degradation, metabolic pathways, peroxisome function, environmental adaptation, and MAPK signaling. These findings provide a foundation for understanding miRNA-mediated regulatory mechanisms in wheat salt tolerance (Qiao et al., 2023).

4 PROSPECTS

miRNAs constitute an important class of small non-coding RNAs in living organisms, playing diverse roles in regulating growth and development, programmed cell death, and metabolism. Their discovery represents a milestone breakthrough in RNA research (Yu et al., 2026). At present, studies on miRNAs are predominantly centered on model plants with well-characterized genomes, whereas research on non-model plants remains comparatively limited. Expanding future investigations into miRNAs in non-model plants, particularly those with scarce genomic information, will be of great significance for broadening the range of both study species and research fields.

Although the functions of certain miRNAs in plant growth and development have been validated, the mechanisms of action for most miRNAs remain poorly understood (Yu et al., 2019). Furthermore, current research tends to emphasize the discovery of novel miRNAs, often at the expense of systematic analysis of regulatory networks. In fact, most miRNAs can regulate multiple target genes, and conversely, a single gene may be targeted by multiple miRNAs, indicating that miRNA-mediated regulation constitutes a complex network (Pandey et al., 2019). Therefore, elucidating the regulatory mechanisms of relevant transcription factors or proteins throughout plant development and life processes, along with clarifying the functions of miRNAs and their intricate network interactions, represents a major challenge in contemporary miRNA research. Moreover, miRNAs play critical roles in regulating gene expression under abiotic and biotic stresses. Accumulating evidence indicates that miRNAs are widely involved in responses to various stresses, including temperature extremes, water deficiency, salinity, heavy metals, and pests and diseases (Gao et al., 2022; Chen et al., 2025). However, current studies remain largely focused on individual miRNAs, with limited systematic integration of the interconnections among them. Future efforts should aim to elucidate the mechanisms by which miRNAs respond to different stresses, clarify how they regulate target gene expression, and construct comprehensive regulatory networks by linking related miRNAs, thereby further refining our understanding of the mechanisms underlying miRNA-mediated stress responses. Such advances will not only provide new perspectives for understanding the biological basis of plant stress tolerance but also point to new directions for crop resistance breeding. With continued in-depth research into the biogenesis, function, and mechanisms of miRNAs, along with ongoing improvements in research methodologies, the biological roles, modes of action, and regulatory pathways of miRNAs in plant development and stress responses will become increasingly clarified.

Contributions

Qiufei Wu: Data curation, Formal analysis, Writing-original draft preparation. **Yajing Dou:** Data curation, Formal analysis, Writing-original draft preparation. **Xiaohui Pan:** Writing-review & editing,. **Xianhai Zeng:** Writing-review & editing. **Lixia Zhou**: Data curation, Conceptualization, Investigation, Supervision. All authors have read and agreed to the published version of the manuscript.

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