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Role of miRNAs and siRNAs in biotic and abiotic stress responses of plants

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Abstract

Small, non-coding RNAs are a distinct class of regulatory RNAs in plants and animals that control a variety of biological processes. In plants, several classes of small RNAs with specific sizes and dedicated functions have evolved through a series of pathways. The major classes of small RNAs include microRNAs (miRNAs) and small interfering RNAs (siRNAs), which differ in their biogenesis. miRNAs control the expression of cognate target genes by binding to reverse complementary sequences, resulting in cleavage or translational inhibition of the target RNAs. siRNAs have a similar structure, function, and biogenesis as miRNAs but are derived from long double-stranded RNAs and can often direct DNA methylation at target sequences. Besides their roles in growth and development and maintenance of genome integrity, small RNAs are also important components in plant stress responses. One way in which plants respond to environmental stress is by modifying their gene expression through the activity of small RNAs. Thus, understanding how small RNAs regulate gene expression will enable researchers to explore the role of small RNAs in biotic and abiotic stress responses. This review focuses on the regulatory roles of plant small RNAs in the adaptive response to stresses.

Keywords

small RNA; miRNA; siRNA; biotic; abiotic; stress responses; plant

1 Introduction

Small non-coding RNAs, which consist of 20–24 nucleotides (nt), have been increasingly investigated as important regulators of protein-coding gene expression; these small RNAs function by causing either transcriptional gene silencing (TGS) or post-transcriptional gene silencing (PTGS) [1]. They were first reported in the nematode *Caenorhabditis elegans* [2] and are responsible for the phenomenon known as RNA interference (RNAi), co-

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suppression, gene silencing, or quelling [3–6]. Shortly after these reports were published, researchers demonstrated that PTGS in plants is correlated with the activity of small RNAs [7]. These small RNAs regulate various biological processes, often by interfering with mRNA translation. In plants, two main categories of small regulatory RNAs are distinguished based on their biogenesis and function: microRNAs (miRNAs) and small interfering RNAs (siRNAs). Recently, miRNAs and siRNAs have been shown to be highly conserved, important regulators of gene expression in both plants and animals [8, 9]. The modes of action by which small RNAs control gene expression at the transcriptional and posttranscriptional levels are now being developed into tools for molecular biology research. One important goal of this research is to determine how a stress affects small RNAs and how small RNAs in turn regulate plant responses to a stress.

Plants have evolved sophisticated mechanisms to cope with a variety of environmental stresses. Many plant genes are regulated by stresses such as drought, salt, cold, heat, light, and oxidative stress [10, 11]. Recent evidence indicates that plant miRNAs and siRNAs play a role in biotic and abiotic stress responses [12–14]. The first indication for such roles came from bioinformatics/in silico analysis of miRNAs and their target genes, and cloning of miRNAs from stress-treated *Arabidopsis thaliana* plants, which revealed new miRNAs that had not been previously cloned from plants grown in unstressed conditions [15, 16]. Understanding small RNA-guided stress regulatory networks can provide new insights for the genetic improvement of plant-stress tolerance. Many studies have also revealed complexity and overlap in plant responses to different stresses, and understanding this complexity and overlay will likely lead to new ways to enhance crop tolerance to diseases and environmental stresses. Researchers recently demonstrated that manipulation of miRNA/siRNA-guided gene regulation can help in the engineering of stress-resistant plants [17–19]. In this review, we consider what are currently known and unknown about roles of miRNAs and siRNAs in plant responses to biotic and abiotic stresses. The responses of small RNAs to different types of stresses are discussed in detail.

2 Classes of small RNAs

Independent approaches combining traditional cloning, computational prediction, and high throughput sequencing of small RNA libraries have identified several classes of small RNAs with specific sizes and functions in plants. These classes of small RNAs include miRNAs, repeat-associated small interfering RNAs (ra-siRNAs), natural antisense transcript-derived small interfering RNAs (nat-siRNAs), trans-acting small interfering RNAs (ta-siRNAs), heterochromatic small interfering RNAs (hc-siRNAs), secondary transitive siRNA, primary siRNAs, and long small interfering RNAs (lsiRNAs) [20–22]. The biogenesis and functions of most of these small RNA classes have been well characterized in the model plant *Arabidopsis thaliana*. In general, small RNAs are generated from at least partially double-stranded RNA precursors by the action of ribonuclease III-like Dicer proteins (DCL) [23]. The small RNA duplexes generated by DCL activity have a characteristic 2-nucleotide overhang at the 3' end because of an offset cutting of the DCLs. In plants, these 3' overhangs are stabilized by 2'-O-methylation [24]. Generation of lsiRNAs depends on the DCL and ARGONAUTE (AGO) subfamily protein AGO7. This differs from the generation of the 25- to 31-nt animal PIWI interacting RNAs, which are independent of the Dicer and AGO subfamily protein. Only one strand of the processed small RNA duplex subsequently associates with an RNA-induced silencing complex (RISC) that scans for nucleic acids complementary to the loaded small RNA to execute its function [25, 26]. In plants, small RNAs act in gene silencing by mediating RNA slicing [27], translational repression [28], and histone modification and DNA methylation [29, 30]. The first two mechanisms control gene expression posttranscriptionally, whereas the latter two affect gene expression at the transcriptional level (Fig. 1).

2.1 miRNAs

2.1.1 Biogenesis of miRNAs—miRNAs are small regulatory RNAs of 20–22 nt that are encoded by endogenous *MIR* genes. Their primary transcripts form precursor RNAs, which have a partially double-stranded stem-loop structure and which are processed by DCL proteins to release mature miRNAs [26]. In the miRNA biogenesis pathway, primary miRNAs (pri-miRNAs) are transcribed from nuclear-encoded *MIR* genes by RNA polymerase II (Pol II) [31] leading to precursor transcripts with a characteristic hairpin structure (Fig. 1A). In plants, the processing of these pri-miRNAs into pre-miRNAs is catalyzed by DCL1 and assisted by HYPONASTIC LEAVES 1 (HYL1) and SERRATE (SE) proteins [26]. The pre-miRNA hairpin precursor is finally converted into 20- to 22-nt miRNA/miRNA* duplexes by DCL1, HYL1, and SE. The duplex is then methylated at the 3' terminus by HUA ENHANCER 1 (HEN1) and exported into the cytoplasm by HASTY (HST1), an exportin protein [32, 33]. In the cytoplasm, one strand of the duplex (the miRNA) is incorporated into an AGO protein, the catalytic component of RISC, and guides RISC to bind to cognate target transcripts by sequence complementarity (Fig. 1A). In addition to the control of targets at the posttranscriptional level, miRNAs regulate gene expression by causing epigenetic changes such as DNA and histone methylation [29, 34, 35].

Functional analysis of conserved miRNAs revealed their involvement in multiple biological and metabolic processes in plants. They regulate various aspects of developmental programs including auxin signaling, meristem boundary formation and organ separation, leaf development and polarity, lateral root formation, transition from juvenile-to-adult vegetative phase and from vegetative-to-flowering phase, floral organ identity, and reproduction. They also regulate plant responses to biotic and abiotic stresses, and the miRNA pathway itself (Table 1).

2.1.2 Role of miRNAs in plant stress responses—Environmental stress causes plants to over- or under-express certain miRNAs or to synthesize new miRNAs to cope with stress. Several stress-regulated miRNAs have been identified in model plants under various biotic and abiotic stress conditions, including nutrient deficiency [36], drought [37–39], cold [40], salinity [37, 41], bacterial infection [42], UV-B radiation [43], and mechanical stress [44]. Although stress regulation may imply a potential function of the regulated miRNA in stress responses, it is obvious that the fact that a miRNA is differentially regulated in response to an environmental stress does not necessarily mean that the miRNA is involved in stress adaptation responses.

In a recent report, the levels of 117 miRNAs under salinity, drought, and low-temperature conditions were analyzed using miRNA chips representing nearly all known miRNAs identified in *Arabidopsis* [37]. Seventeen stress-inducible miRNAs were detected, and the results were confirmed by detecting their expression patterns and analyzing the *cis*-regulatory elements in their promoter sequences [37]. Jones-Rhoades and Bartel [15] identified novel *Arabidopsis* miRNAs that were predicted to target genes such as superoxide dismutases, laccases, and ATP sulfurylases (APS). The expression of one particular miRNA (*miR395*) was increased upon sulphate starvation, showing that miRNAs can be induced by environmental factors and not only by developmental processes. *miR395* targets the genes that encode ATP sulfurylases APS1, APS3, and APS4. These enzymes catalyze the first step of inorganic sulfate assimilation [15, 16]. Sunkar and Zhu [16] constructed a library of small RNAs from *Arabidopsis* seedlings exposed to different abiotic stresses including cold, dehydration, high salt, and abscisic acid (ABA), and identified several new miRNAs that are responsive to abiotic stress [16]. For example, *miR393* was upregulated by cold, dehydration, salinity, and ABA treatments; *miR397b* and *miR402* were slightly upregulated

by general stress treatments while *miR319c* was induced by cold but not by the other treatments; *miR389a*, however, was downregulated by all of the stress treatments. The results indicated that stress-induced miRNAs target negative regulators of stress responses or positive regulators of processes that are inhibited by stresses and that several of the newly identified miRNAs exhibit tissue- or developmental stage-specific expression patterns. More recently, genome-wide profiling and analysis of miRNAs was carried out in drought-challenged rice [39]. Lu et al. [44] also identified 48 miRNA sequences from the *Populus* genome and found that most of these *Populus* miRNAs target developmental and stress/defense-related genes. The authors also found that plant miRNAs can be induced by mechanical stress and may function in critical defense systems for structural and mechanical fitness [44].

A number of miRNAs have been linked to biotic stress responses in plants, and the role of these miRNAs in plants infected by pathogenic bacteria, viruses, nematodes, and fungi has been reported [12, 45]. Moreover, miRNAs are also important in regulating plant–microbe interactions during nitrogen (N) fixation by *Rhizobium* and tumor formation by *Agrobacterium* [45]. Additionally, Mishra et al. [46] observed a significant increase in the GC content of stress-regulated miRNA sequences, which further supports the view that miRNAs act as ubiquitous regulators under stress conditions. GC content may also be considered a critical parameter for predicting stress-regulated miRNAs in plants [46].

It is noteworthy that some of the regulations were seen from only a single species and may not be applicable to other species. In fact, it remains to be seen whether some of the observations may even be confirmed by an independent method or reproducible from independent experiments with the same species.

2.1.2.1 miRNAs involved in ABA-mediated stress responses: The phytohormone ABA is involved in plant responses to environmental stresses. The first indication that miRNAs may be involved in ABA-mediated responses came from observations of ABA hypersensitivity in an *Arabidopsis* mutant containing a “pleiotropic recessive *Arabidopsis* transposon insertion mutation,” *hyl1* [47]. Recently, two research groups independently found that either ABA or gibberellin (GA) treatment regulated *miR159* expression [16, 48] and controlled floral organ development [48]. In germinating *Arabidopsis* seeds, *miR159* was upregulated in ABA-treated seedlings [49]. Sunkar and Zhu [16] reported that the expression of *miR393*, *miR397b* and *miR402* was upregulated by ABA treatment. In contrast, *miR389a* appears to be downregulated by ABA [16]. Other studies in *Arabidopsis* have also reported upregulation of *miR160* [50] and *miR417* [51] and downregulation of *miR169* [52] and *miR398* [53] in response to ABA. In rice, *miR319* was upregulated, whereas *miR167* and *miR169* were downregulated in ABA-mediated responses [54]. In *Phaseolus vulgaris*, *miR159.2*, *miR393*, and *miR2118* were induced under ABA treatments whereas *miRS1*, *miR1514*, and *miR2119* were moderately upregulated in response to ABA (Fig. 2) [55]. The dependence of DNA methylation on miRNA levels was also shown for an ABA-responsive *PpbHLH-miR1026* regulon in *Physcomitrella patens*. ABA application caused an increase of *miR1026* and a decrease of its *PpbHLH* target RNA [29].

2.1.2.2 miRNAs in response to drought and salt stress: Studies with *Arabidopsis* have revealed a general role for miRNAs in drought and salt responses (Fig. 2). Several stress-related miRNAs were identified based on the sequencing of a library of small RNAs isolated from *Arabidopsis* seedlings exposed to various stresses [16]. Recently, miRNAs such as *miR168*, *miR171*, and *miR396* were found to be responsive to high salinity, drought, and cold stress in *Arabidopsis*, thus supporting the hypothesis of a role for miRNAs in the adaptive response to abiotic stress [37]. miRNA-expression profiling under drought stress has now been performed in *Arabidopsis*, rice, and *Populus trichocarpa*. In *Arabidopsis*,

miR396, *miR168*, *miR167*, *miR165*, *miR319*, *miR159*, *miR394*, *miR156*, *miR393*, *miR171*, *miR158*, and *miR169* were shown to be drought responsive [37]. The upregulation of *miR393*, *miR319*, and *miR397* in response to dehydration in *Arabidopsis* has been reported [16]. In rice, *miR169g* was strongly upregulated by drought while *miR393* was transiently induced by drought [38]. Genome-wide profiling and analysis of miRNAs were carried out in drought-challenged rice across a wide range of developmental stages, from tillering to inflorescence formation, using a microarray platform [39]. The results indicated that 16 miRNAs (*miR156*, *miR159*, *miR168*, *miR170*, *miR171*, *miR172*, *miR319*, *miR396*, *miR397*, *miR408*, *miR529*, *miR896*, *miR1030*, *miR1035*, *miR1050*, *miR1088*, and *miR1126*) were significantly downregulated in response to drought stress. Conversely, 14 miRNAs (*miR159*, *miR169*, *miR171*, *miR319*, *miR395*, *miR474*, *miR845*, *miR851*, *miR854*, *miR896*, *miR901*, *miR903*, *miR1026*, and *miR1125*) were significantly upregulated under drought stress. Some miRNA gene families, such as *miR171*, *miR319*, and *miR896*, were identified in both down- and upregulated groups, and nine miRNAs (*miR156*, *miR168*, *miR170*, *miR171*, *miR172*, *miR319*, *miR396*, *miR397*, and *miR408*) showed opposite expression to that observed in drought-stressed *Arabidopsis* [39]. In *Populus*, *miR171l-n*, *miR1445*, *miR1446a-e*, and *miR1447* were found to be drought-responsive [56]. In *Phaseolus vulgaris*, *miRS1*, *miR1514a*, and *miR2119* showed a moderate but clear increase in accumulation upon drought treatment, while the accumulation was greater for *miR159.2*, *miR393*, and *miR2118* in response to the same treatment [55]. In *Medicago truncatula*, *miR169* was downregulated only in the roots, and *miR398a,b* and *miR408* were strongly upregulated in both shoots and roots under drought stress [57]. More recently, miRNA expression patterns of drought-resistant wild emmer wheat (*Triticum turgidum* ssp. *Dicoccoides*) in response to drought stress were investigated using a plant miRNA microarray platform [58]. Expression levels were detected for 205 miRNAs in control and 438 miRNAs in drought-stressed leaf and root tissues. The following 13 miRNAs were found to be differentially regulated in response to drought stress: *miR1867*, *miR896*, *miR398*, *miR528*, *miR474*, *miR1450*, *miR396*, *miR1881*, *miR894*, *miR156*, *miR1432*, *miR166*, and *miR171* [58]. Similarly, *miR474*, which targets proline dehydrogenase (PDH), was recently shown to be upregulated during drought stress in maize [59].

Approximately 6% of the total arable land is affected by excess salt [60]. Numerous genes and pathways in plants are affected by salt stress [11]. Several differentially regulated miRNAs have been identified in salt-stressed plants. In *Arabidopsis*, *miR156*, *miR158*, *miR159*, *miR165*, *miR167*, *miR168*, *miR169*, *miR171*, *miR319*, *miR393*, *miR394*, *miR396*, and *miR397* were upregulated in response to salt stress (Fig. 2), while the accumulation of *miR398* was decreased [37]. In *Phaseolus vulgaris*, Arenas-Huerta et al. [55] observed increased accumulation of *miRS1* and *miR159.2* in response to NaCl addition (Fig. 2). In *Populus trichocarpa*, *miR530a*, *miR1445*, *miR1446a-e*, *miR1447*, and *miR171l-n* were downregulated, whereas *miR482.2* and *miR1450* were upregulated during salt stress [56]. Later, *miR169g* and another member of the *miR169* family, *miR169n*, were reported to be induced by high salinity [61]. The authors found a *cis*-acting ABA-responsive element (ABRE) in the upstream region of *miR169n*, which suggested that *miR169n* may be ABA-regulated. Both *miR169g* and *miR169n* targeted Nuclear factor Y subunit A (NF-YA), a factor that was previously shown to be downregulated in drought-affected wheat leaves [62]. Recently, a report used microarray experiments to explore the miRNA profile in a salt-tolerant and a salt-sensitive line of maize (*Zea mays*) [63]; the results indicated that members of the *miR156*, *miR164*, *miR167*, and *miR396* families were downregulated, while *miR162*, *miR168*, *miR395*, and *miR474* families were upregulated in salt-shocked maize roots (Fig. 2).

2.1.2.3 miRNAs in response to cold and heat stress: The expression of miRNAs in cold stress has been examined in *Arabidopsis* [16, 37], *Populus* [56], and *Brachypodium* [64].

miR397 and *miR169* were upregulated in all three species, and *miR172* was upregulated in *Arabidopsis* and *Brachypodium*. In addition to these miRNAs, several miRNAs (*miR165/166*, *miR393*, *miR396*, and *miR408*) were induced under cold stress in *Arabidopsis* (Fig. 2), while other miRNAs (*miR156/157*, *miR159/319*, *miR164*, *miR394*, and *miR398*) showed either transient or mild regulation under cold stress [16, 37]. In *Populus*, *miR168a,b* and *miR477a,b* were upregulated while *miR156g-j*, *miR475a,b*, and *miR476a* were downregulated under cold stress (Fig. 2) [56]. Wheat miRNAs showed differential expression in response to heat stress; by using Solexa high-throughput sequencing, Xin et al. [65] cloned the small RNAs from wheat leaves treated by heat stress. Among the 32 miRNA families detected in wheat, nine conserved miRNAs were putatively heat responsive. For example, *miR172* was significantly decreased, and miRNAs (including *miR156*, *miR159*, *miR160*, *miR166*, *miR168*, *miR169*, *miR393* and *miR827*) were upregulated under heat stress (Fig. 2).

2.1.2.4 miRNAs in response to hypoxia and oxidative stress: Anaerobic or low-oxygen stress (hypoxia) interferes with mitochondrial respiration [66]. Hypoxia induces massive changes in the transcriptome and a switch from aerobic respiration to anaerobic respiration [67]. Recent studies suggested that miRNAs are involved in plant responses to hypoxia [68, 69]. Several miRNAs were differentially regulated in seedlings that were submerged; early during submergence, *Zm-miR166*, *Zm-miR167*, *Zm-miR171*, *Os-miR396*, and *Zm-miR399* were induced but *Zm-miR159*, *At-miR395*, *Pt-miR474*, and *Os-miR528* were downregulated (Fig. 2) [69]. Similarly, high-throughput sequencing was carried out on small RNA libraries from hypoxia-treated and control root tissues in *Arabidopsis* [68]. The abundances of 19 miRNAs families significantly changed in response to hypoxia, and their abundances were inversely correlated with those of their specific mRNA targets [68]. Expression levels of *miR156g*, *miR157d*, *miR158a*, *miR159a*, *miR172a,b*, *miR391*, and *miR775* increased under hypoxia. The involvement of miRNAs in plant response to hypoxia was further confirmed in experiments with a chemical that inhibits mitochondrial respiration and therefore mimics hypoxia [68].

Reactive oxygen species (ROS) are inherent to plants because ROS are constantly produced by aerobic processes in chloroplasts, mitochondria, and peroxisomes [70]. Elevated levels of ROS are often associated with plant stress such as high intensity light, UV radiation, temperature extremes, heavy metals, drought stress, salt stress, and mechanical stress [70]. Superoxide in plants are converted into molecular oxygen and hydrogen peroxide by superoxide dismutases (SODs). Cu-Zn SODs are encoded by *CSD1*, *CSD2*, and *CSD3* in *Arabidopsis* [19]. *miR398* was predicted to target *CSD1* and *CSD2* [15, 71], and Sunkar et al. [19] confirmed these targets and discovered that *miR398* is downregulated under oxidative stress. Downregulation of *miR398* is accompanied by an accumulation of *CSD1* and *CSD2* transcripts [19]. This accumulation did not result from stress-related transcriptional induction of the *Cu/Zn SOD* genes, but rather resulted from the relaxation of *miR398*-directed cleavage [19]. Furthermore, transgenic *Arabidopsis* plants overexpressing a *miR398*-resistant form of *CSD2* accumulated more *CSD2* mRNA than plants overexpressing a regular *CSD2* and were consequently much more tolerant to high intensity light, heavy metals, and other oxidative stressors [19]. Under stress conditions, the negative regulation of *CSD1* and *CSD2* by *miR398* is relieved, which leads to the increased expression of *CSD1* and *CSD2* and therefore reduced accumulation of the highly toxic superoxide free radicals [19]. *miR398* was induced under Cu^{2+} -limited conditions to relocate the Cu^{2+} ion from *CSD1* and *CSD2* for synthesis of more essential proteins and processes [72]. A genome-wide study of H_2O_2 -regulated miRNAs from rice seedlings indicated that expression of some miRNAs is regulated in response to oxidative stress [73]. The authors identified seven H_2O_2 -responsive miRNAs (*miR169*, *miR397*, *miR528*, *miR827*, *miR1425*, *miR319a.2*, and *miR408-5p*), i.e., miRNAs that were expressed differentially in H_2O_2 -treated and control

samples; *miR169*, *miR397*, *miR827*, and *miR1425* were upregulated while *miR528* was downregulated by H₂O₂-treatments [73].

2.1.2.5 miRNAs in response to UV-B radiation: A computational approach was used to identify *Arabidopsis* miRNAs induced by UV-B radiation [43]. Of the 21 miRNAs belonging to 11 miRNA families identified in that study, the following were predicted to be upregulated under UV-B stress: *miR156/157*, *miR159/319*, *miR160*, *miR165/166*, *miR167*, *miR169*, *miR170/171*, *miR172*, *miR393*, *miR398*, and *miR401* [43]. Some of the same miRNA families that were predicted to be upregulated by UV-B radiation in *Arabidopsis* (*miR156*, *miR160*, *miR165/166*, *miR167*, *miR398*, and *miR168*) were found to be upregulated by UV-B radiation in *Populus tremula* (Fig. 2) [74]. Interestingly, three families (*miR159*, *miR169*, and *miR393*) that were predicted to be upregulated in *Arabidopsis* were downregulated in *Populus tremula*, suggesting that some responses to UV-B radiation stress may be species specific [74].

2.1.2.6 miRNAs and nutrient homeostasis: miRNAs are involved in plant responses to nutrient stress. According to recent studies with *Arabidopsis*, *miR399*, *miR395*, and *miR398* are induced in response to phosphate-, sulfate-, and copper-deprived conditions, respectively [15, 19, 72, 75, 76]. Phosphate is required for the synthesis of nucleic acids and membrane lipids and is frequently a limiting nutrient for plant growth. Phosphate homeostasis is partially controlled through *miR399*, which targets a gene encoding a putative ubiquitin-conjugating enzyme (UBC24) [15, 16]. *miR399* is upregulated in low phosphate-stressed plants (Fig. 2) [36, 76, 77] and is not induced by other common stresses, whereas its target, *UBC24* mRNA, was reduced primarily in roots of plants exposed to low-phosphate stress. It is likely that an MYB transcription factor, PHOSPHATE STARVATION RESPONSE 1 (*PHR1*), is involved in *miR399* expression. *PHR1* is expressed in response to phosphate starvation and positively regulates a group of phosphate-responsive genes by binding to GNATATNC *cis*-elements [78–80]. This *cis*-element has been found upstream of all known *miR399* genes in *Arabidopsis* [76, 78]. Furthermore, *phr1* mutants show a significant decline in *miR399* induction under phosphate stress [76, 78]. *miR399* has been isolated from phloem, and its abundance in phloem increases upon phosphate starvation [81, 82]. These studies strongly suggest that *miR399* acts as a phosphate starvation signal that is translocated from shoot to root where it promotes phosphate uptake by downregulating *PHO2*, which encodes E2-UBC24 [75, 76]. miRNAs themselves can be subject to posttranscriptional regulation, as revealed by the discovery of the INDUCED BY PHOSPHATE STARVATION1 (*IPSI*) gene, which acts as a target mimic to control *miR399* action [83]. *IPSI* has imperfect sequence complementarity to *miR399*. The complementarity is interrupted by a mismatched loop at the expected miRNA cleavage site. Because of this complementarity interruption, the *IPSI* RNA sequesters *miR399* and prevents it from causing degradation of *PHO2* mRNA [83].

Sulfur (S) is one of the essential macronutrients and is available in the form of sulfate in the soil. *miR395* targets both ATP sulfurylases (*APSs*) and the sulfate transporter *AST68* [9, 15]. Sulfate deprivation induces the expression of *miR395* (Fig. 2), with a concomitant decrease in transcript levels of *APS1* [15]. The abundance of *miR395* in the phloem increases for *Brassica* plants deprived of S, and the increase was much stronger in the phloem than in the root, stem, or leaf tissue [81, 84].

The plant micronutrient copper (Cu) is essential for photosynthesis, oxidative responses, and other physiological processes [85]. *miR398* is a key regulator of Cu homeostasis. When Cu is limiting, the level of *miR398* increases (Fig. 2), and this reduces the allocation of Cu to CSDs (*CSD1* and *CSD2*) and therefore makes Cu available for other essential processes [72, 86]. In higher plants, the Cu/Zn-SODs are replaced by Fe-SODs [87]. In *Brassica* under Cu

deprivation, *miR398* is upregulated not only in leaf, stem, and root tissue but also in phloem sap [84]. According to more recent research, *Brassica* phloem sap contains a specific set of small RNAs that are distinct from those in leaves and roots, and the phloem responds specifically to stress [81]. Upon S and Cu deficiencies, the phloem sap reacts with an increase of the same miRNAs that were earlier characterized in other tissues, while no clear positive response to iron was observed. However, iron led to a reduction of copper- and phosphate-responsive miRNAs. Grafting experiments also indicated that *miR395* and *miR399* are phloem-mobile, suggesting that such translocatable miRNAs might be candidates for information-transmitting molecules [81].

Cadmium (Cd) is one of the most toxic metals in agricultural soils. Under high concentrations of Cd or other heavy metals, plants encounter cation imbalances that result in many physiological and biochemical disorders. Recent studies identified a set of conserved and non-conserved miRNAs that are differentially regulated in response to heavy metals in rice [88], *Medicago truncatula* [89], *Brassica napus* [90], and *Arabidopsis* [91]. These results suggest that miRNAs help regulate plant responses to heavy metal stress in addition to other abiotic stresses. In *Brassica napus*, expression of miRNAs shows different responses to sulfate deficiency and Cd exposure (Fig. 2) [90]. *miR160* was transcriptionally downregulated by sulphate deficiency and by Cd exposure but *miR164b* and *miR394a,b,c* in roots and stems were upregulated by sulfate deficiency. Similarly, treatment with Cd induced expression of *miR164b* and *miR394a,b,c* in all tissues (Fig. 2), except that *miR164b* was downregulated in leaves [90]. *miR156a* and *miR167b* in roots and *miR156a* and *miR167c* in leaves were upregulated under sulfate deficiency. In contrast, *miR167a* and *miR168* in roots and *miR167a*, *miR167b*, and *miR168* in leaves were downregulated [90]. Under Cd stress, most *B. napus* miRNAs were induced. Notably, *miR156a*, *miR167a*, and *miR167c* in roots and *miR167a* and *miR167c* in leaves were strongly upregulated. *miR393* expression in leaves was also upregulated by Cd exposure [90]. Xie et al. [92] reported that Cd exposure increased the expression of *miR156*, *miR171*, *miR393*, and *miR396a* in *B. napus* roots but the accumulation of the transcripts for these miRNAs was suppressed [92].

Huang et al. [88] constructed a library of small RNAs from rice seedlings that were exposed to toxic levels of Cd. In most cases, the rice miRNAs showed different patterns of expression in leaves and roots. *miR601*, *miR602*, and *miR603* in roots were upregulated while *miR602* and *miR606* in leaves and *miR604* in roots were downregulated by Cd exposure (Fig. 2). The rest of rice miRNAs, such as *miR601* in leaves and *miR605* and *miR606* in roots, do not appear to be regulated by Cd stress [88]. Cd, mercury (Hg), and aluminum (Al) also regulated the expression of miRNAs in *Medicago truncatula* [89]. In that study, *miR171*, *miR319*, *miR393*, and *miR529* were upregulated in response to Hg, Cd, and Al, and *miR319* showed weak constitutive expression in leaves; it was upregulated by Cd and Al but was not affected by Hg. Similarly, expression of *miR393* was not affected by Al but was slightly upregulated by Hg and Cd. In contrast, *miR166* and *miR398* were downregulated by Hg, Cd, and Al exposure [89].

Rice *miR1432* and *miR444d*, which were identified by high throughput sequencing, are predicted to target a calmodulin-binding protein and an EF-hand protein, respectively, which suggests a role for these miRNAs in calcium signaling [93]. miRNAs also affect the growth of specific cells in roots under N-limiting conditions [94]. In the presence of N, *miR167a* was repressed (Fig. 2) and its target, *ARF8*, was induced in the pericycle, resulting in the initiation of many lateral roots. When N is limiting, lateral root extension is stimulated, and this permits roots to search for N in the soil further from the plant [94]. The *Arabidopsis* *miR169* was strongly downregulated, whereas its targets, *NFYA* was strongly induced by nitrogen N starvation. Analysis of the expression of *miR169* precursors showed that *miR169a* was substantially downregulated in both roots and shoots by N starvation.

Accumulation of the *NFYA* family members was suppressed in transgenic *Arabidopsis* with constitutive expression of *miR169a* [95]. Several *pri-miR169* species as well as *pri-miR398a* were found to be repressed during N limitation, whereas during P limitation, *pri-miR778*, *pri-miR827*, and *pri-miR399* species were induced and *pri-miR398a* was repressed [96]. Bioinformatic analysis of small RNA sequences with a modified miRDeep algorithm led to the identification of the novel P limitation-induced *miR2111*, which is encoded by two loci in the *Arabidopsis* genome. Furthermore, *miR2111*, *miR169*, a *miR827*-like sequence, and the abundances of several miR star strands were found to be strongly dependent on P or N status in rapeseed (*Brassica napus*) phloem sap, flagging them as candidate systemic signals [96].

Expression of miRNAs also are affected by Fe deficiency, in *Arabidopsis miR169b*, *miR169c*, *miR172c*, *miR172d*, *miR173* and *miR394b* in roots and *miR169c*, *miR172c*, *miR172d*, *miR173*, *miR394a* and *miR394b* in shoots was initially up-regulated and then down-regulated during the period of Fe deficiency. These results indicate that the cloned miRNAs respond to Fe deficiency [97].

2.1.2.7 miRNAs and mechanical stress: Plants experience mechanical stress, which is also referred to as static and dynamical stress when branches or stems are bent by wind, gravity, or other external forces. In a study of *Populus trichocarpa*, Pt-miRNA transcript levels were compared in tension-stressed or compression-stressed xylem with those in unstressed xylem [44]. *miR156*, *miR162*, *miR164*, *miR475*, *miR480*, and *miR481* were downregulated but *miR408* was upregulated by tension and compression (Fig. 2). *miR160* and *miR172* were downregulated only in compressed tissue while *miR168* was upregulated only in tissue under tension stress. These results show that plant miRNAs can be regulated by mechanical stress and may function in one of the critical defense systems for structural and mechanical fitness [44].

2.1.2.8 miRNAs and biotic stress: In mammals, several examples of miRNA-mediated inhibition or enhancement of viral infection have been reported [98]. In plants, *Arabidopsis miR393* was the first miRNA reported to play a role in plant antibacterial *PTI* (pattern triggered immunity) by regulating the auxin signaling pathway. *miR393* was induced by a bacterial PAMP (pattern associated molecular pattern) peptide *flg22* post-treatment [42]. Using small RNA-expression profiling on *Arabidopsis* leaves collected at 1 and 3 h post-inoculation (hpi) with *Pst DC3000 hrcC*, Fahlgren et al. [99] identified three miRNAs (*miR160*, *miR167*, and *miR393*) that were highly induced and one that was downregulated (*miR825*) after infection (Fig. 2). The role of miRNAs in plant basal defense was further supported by the finding that *Arabidopsis* miRNA-deficient mutants *dcl1* and *hen1* showed enhanced growth of the bacterium *Pst DC3000 hrcC* and several bacteria strains that are non-pathogenic to *Arabidopsis*, including *Pseudomonas syringae* pv. *phaseolicola*, *P. fluorescens*, and *E. coli* [100]. Navarro et al. [100] showed that the induction of *flg22*-responsive *pri-miR393* and *pri-miR396* in *Arabidopsis* was suppressed by the virulent strain *Pst DC3000* but not by the *TTSS* mutant strain *Pst DC3000 hrcC*. *miR398* in *Arabidopsis* was downregulated by infection of with *Pst (avrRpm1)* and *Pst (avrRpt2)* but was unaffected by *Pst DC3000* infiltration [101]. Also in *Arabidopsis*, *miR156* and *miR164* were induced by infection with the virus *TYMV p69* and were induced in transgenic *Arabidopsis* plants expressing the viral silencing suppressor *PI/HC-Pro* [102]. In tobacco, *miR156*, *miR160*, and *miR164* were induced after virus infection [100]. Upon virus infection, new miRNAs (*miR158* and *miR1885*) were identified in *Brassica*; *miR158* and *miR1885* were specifically induced by *turnip mosaic virus* but not by *cucumber mosaic virus* or *tobacco mosaic virus* [104].

Various miRNA families were differentially expressed in response to infection by the rust fungus *Cronartium quercuum* f. sp. *fusiforme*, which causes fusiform rust disease in pines; 10 of 11 miRNA families were downregulated, including seven that were pine specific [102]. For example, *miR156* and *miR160* were repressed in pine stems infected with the rust fungus (Fig. 2) [105]. More recent data indicate that miRNAs may affect the response of wheat (*Triticum aestivum* L.) to powdery mildew infection; some of the conserved miRNAs showed differential expression patterns in response to powdery mildew infection: *miR156*, *miR159*, *miR164*, *miR171*, and *miR396*, were downregulated and *miR393*, *miR444*, and *miR827*, were upregulated, respectively [65]. In addition, a negative correlation between miRNA abundance and their targets was observed in roots infected by the plant-parasitic nematode *Heterodera schachtii*, which confirmed that miRNAs are involved in plant–nematode interactions [103]. In response to *H. schachtii* infection, *miR161*, *miR164*, *miR167a*, *miR172c*, *miR396a,b*, and *miR398a* were downregulated (Fig. 2). Expression of *miR156* and *miR160* was substantially repressed in the galled stem (Fig. 2).

miRNAs also play critical and diverse roles in symbiotic N fixation. To identify potential regulators early in nodule development, Subramanian et al. [107] inoculated soybean (*Glycine max*) roots with *Bradyrhizobium japonicum* and identified differentially expressed miRNAs. For example, *miR168* and *miR172* were upregulated at 1 or 3 hpi but returned to basal levels by 12 hpi; *miR159* and *miR393* were upregulated by 3 hpi and continued to maintain these levels to 12 hpi; *miR160* and *miR169* were downregulated in response to rhizobia. Also, rhizobial infection changed the levels of *miR160*, *miR393*, *miR164*, and *miR168*, which target *ARFs* (*ARF10*, *ARF16*, and *ARF17*), *TIR1*, *NAC1*, and *AGO1*, respectively (Fig. 2). Li et al. [108] examined the gene expression levels of six families of novel miRNAs and investigated their functions in nodule development in soybean. The results suggested that *miR482*, *miR1512*, and *miR1515* might have specific and important functions during soybean nodulation [108]. Cloning and sequencing of miRNAs from functional nodules of soybean revealed conserved miRNAs (*miR167*, *miR172*, *miR396*, and *miR399*), whereas four other families had sequences homologous to *Gm-miR1507*, *Gm-miR1508*, *Gm-miR1509*, and *Gm-miR1510*, which play a role in N fixation [109]. In *Medicago truncatula*, the functions during nodulation have been studied for two conserved miRNAs; *miR169* regulated *MtHAP2-1*, which altered nodule development [110], while overexpression of *miR166* reduced the number of symbiotic nodules and lateral roots [111].

2.2 siRNAs

2.2.1 Biogenesis of siRNAs—siRNAs are generated from perfectly double-stranded RNAs that can originate from different sources such as RNAs transcribed from inverted repeats, natural *cis*-antisense transcript pairs, the action of RNA-dependent RNA polymerases (RDRs) which convert single-stranded RNA into dsRNA, the replication of RNA viruses, and regions of the genome rich in retroelements (Fig. 1B). The dsRNA is cleaved into 21- to 24-nt siRNAs by DCLs proteins, and the size of the released siRNAs depends on the specific catalytic activity of the respective DCL protein. dsRNA is usually cleaved by multiple DCL proteins, thereby generating siRNA classes with different sizes. Like miRNAs, siRNAs are loaded into AGO protein-containing RISC that guides target regulation at the posttranscriptional level or at the transcriptional level through a pathway termed RNA-directed DNA methylation (RdDM) (Fig. 1B).

2.2.2 Role of siRNAs in plant stress responses

2.2.2.1 siRNAs and abiotic stress: The first evidence that siRNAs are involved in abiotic stress responses in plants was provided by Sunkar and Zhu [16]. Work on the founding member of nat-siRNAs, which is derived from a natural *cis*-antisense transcript pairs of *SRO5* and *P5CDH* genes, demonstrated an important role of nat-siRNAs in osmoprotection

and oxidative stress management under salt stress in *Arabidopsis* [112]. *P5CDH* is constitutively expressed, whereas *SRO5* is induced by salt stress. Under high salt stress, the 24-nt nat-siRNA corresponding to the *SRO5* mRNA is produced; this nat-siRNA targets the *P5CDH* mRNA for degradation and leads to the production of a population of 21-nt nat-siRNAs. Downregulation of *P5CDH* leads to proline accumulation, which is an important step contributing to the plant's ability to tolerate excess salt [112]. However, reduced *P5CDH* activity also leads to the accumulation of P5C (a toxic metabolic intermediate) and ROS; that accumulation is probably countered by the *SRO5* protein through direct detoxification activity in the mitochondria [112]. The initial induction of *SRO5* mRNA by salt stress may be mediated by oxidative stress that is an inevitable consequence of salt stress. Thus, the *SRO5-P5CDH* nat-siRNAs together with the *P5CDH* and *SRO5* proteins are key components of a regulatory loop controlling ROS production and salt stress response [112].

Abiotic stress responsiveness has also been observed in a pool of *Triticum aestivum* small noncoding RNAs [113]. In wheat seedlings, cold, heat, salt, or drought stresses substantially change the expression of four siRNAs: *siRNA002061_0636_3054.1* is strongly downregulated by heat, salt, and drought; *siRNA 005047_0654_1904.1* is greatly upregulated by cold stress and downregulated by heat, salt, and drought; *siRNA080621_1340_0098.1* is slightly upregulated by cold and downregulated by heat but not by salt and drought; and *siRNA007927_0100_2975.1* is downregulated by cold, salt, and drought but not by heat stress [113].

TasiRNAs are a specialized class of siRNAs that are generated by miRNA processing of a *TAS* gene transcript, resulting in the production of 21-nt RNAs that are phased with respect to the miRNA cleavage site. Four families of *TAS* genes have been identified in *Arabidopsis*, with *TAS1* and *TAS2* transcripts recognized by *miR173*, *TAS3* recognized by *miR390*, and *TAS4* targeted by *miR828* [114]. *TAS1*, *TAS2*, and *TAS3* tasiRNAs all showed increased expression in hypoxia-treated samples in *Arabidopsis* [68]. These changes in tasiRNA levels are reflected in the changes to *TAS*-targeting miRNAs; both *miR173* and *miR390* showed increased expression [68]. Most pentatricopeptide (PPR) repeat-containing proteins (PPRs) targeted by hypoxia-responsive small RNAs are from the P subfamily, which is predicted to localize to the mitochondria [68]. It is likely that some of the observed changes in tasiRNAs are the result of decreased mitochondrial function during hypoxia because *TAS1* is induced by chemical inhibition of the cytochrome and alternative oxidase respiration pathways in the mitochondria. The downregulation of these *PPR* genes may either protect mitochondria during hypoxia or simply reflect a decreased requirement for these gene transcripts.

Stress responses in plants also involve novel long non-protein coding RNAs (npcRNA). In *Arabidopsis*, salt stress resulted in a dramatic increase in *npcRNA60* and *npcRNA536* and a decrease in *npcRNA72* and *npcRNA82* accumulation [115]. In the same study, phosphate deprivation caused substantial upregulation of *npcRNA43* and *npcRNA536*, substantial downregulation of *npcRNA33*, slight upregulation of *npcRNA60*, and slight downregulation of *npcRNA311* [115]. In *Craterostigma plantagineum*, an endogenous siRNA has been identified that is induced during dehydration and that may contribute to desiccation tolerance [116]. The authors describe the T-DNA tagging of a *Craterostigma* gene that induces desiccation tolerance by activating ABA-inducible genes. Activation tagging was used to identify constitutively desiccation tolerant (CDT) callus lines, and this led to the identification of CDT-1 (constitutively desiccation tolerant-1) callus lines. In wild-type *C. plantagineum*, the transcript levels of CDT-1 were upregulated by ABA and dehydration treatments but repressed when rehydrated. Interestingly, sense and antisense siRNAs corresponding to the *CDT-1* locus were detected, suggesting that during stress (ABA or

dehydration) the locus generates small RNAs that may confer stress tolerance through induction of stress-responsive genes [116].

2.2.2.2 siRNAs and biotic stress: The first plant-endogenous siRNA that was found to be involved in plant biotic stress was *nat-siRNAATGB2*, which regulates *R*-gene mediated ETI (effector triggered immunity) [18]. This siRNA is specifically induced by *Pst DC3000* carrying effector *avrRpt2* (Fig. 2). Induction of *nat-siRNAATGB2* represses the expression of its antisense target *PPRL*, a negative regulator of *RPS2*-mediated ETI against *Pst* (*avrRpt2*). A novel class of endogenous siRNA, the lsiRNAs, are 30–40 nt long and are induced by bacterial infection or specific growth conditions, such as cell suspension culture [17]. *AtlsiRNA1* is strongly induced by *Pst* (*avrRpt2*), whereas *AtlsiRNA2*, *AtlsiRNA3*, and *AtlsiRNA4* are moderately induced by infection with various *Pst* strains and/or mock treatment. siRNAs also differentially accumulate in response to *H. schachtii* infection; infection by this nematode caused a substantial increase in expression of *siRNA9*, *siRNA41*, and *siRNA46* but a decrease in expression of *siRNA32* (Fig. 2) [106].

3 Stress-miRNA networks

The spectrum of action by miRNAs seems to be extremely wide and includes various aspects of development, adaptive responses to stresses, and the regulation of the miRNA pathway itself. Most miRNAs do not function independently but rather are involved in overlapping regulatory networks (Table 1). One obvious feature of the adaptation to stress is change in gene expression profiles for genes involved in a broad spectrum of biochemical, cellular, and physiological processes [117]. Under optimal conditions, all resources are used for supporting plant growth and development. Under stress, however, growth and development are stalled, and the resources are mobilized toward adaptive responses to stress. Most conserved miRNAs target mRNAs encoding diverse families of transcription factors. For example, *miR156*, *miR159/319*, *miR160*, *miR166*, and *miR169* target *SBPs*, *MYBs/TCPs*, *ARFs*, *HD-ZIPs*, and the *NFY* subunit, respectively, but *miR168*, *miR393*, *miR395*, and *miR398* target mRNAs encoding AGO1, TIR1, ATS/APS, and CSD1/2, respectively [9, 21]. The level of those conserved miRNAs appears to be regulated during stress, and their target genes appear to be stress regulated as well (Fig. 3), suggesting that plant growth and development are modulated during stress.

The stress-responsive miRNAs could be involved in many pathways that reprogram complex processes of metabolism and physiology. For example, upregulation of *miR395* is involved in sulfur metabolism (Fig. 3); *miR395* targets ATP sulfurylases (APS1, APS3, and APS4), which catalyze the first step of the sulfur assimilation pathway [118]. *miR395* also targets AST68, a low-affinity sulfate transporter [114]. AST68 is implicated in the internal translocation of sulfate from roots to shoots [119]. Thus, *miR395* potentially coordinates changes in sulfate translocation and assimilation. In addition, downregulation of *miR398* during stress enables the mRNA levels of both targets (*CSD1* and *CSD2*) to increase and detoxify ROS (Fig. 3). *miR398* also targets the gene cytochrome *C* oxidase subunit V [15, 16]; further studies are required to determine whether this gene is also part of the oxidative stress network in plants. Downregulation of *miR169a* and *miR169c* allows expression of the mRNA of *NFYA5*, which is important for drought responses in *Arabidopsis* [52]. Researchers have hypothesized that *NFYA5* expressed in guard cells controls stomatal aperture, whereas *NFYA5* expressed in other cells may contribute to the expression of stress-associated genes (Fig. 3). Because AGO1 is a key miRNA pathway regulator, the difference in *AGO1* mRNA expression under salt and drought stress may induce further changes of numerous miRNA activities (Fig. 3). In addition, AGO1 and miR168 seem to be transcriptionally coregulated, allowing *AGO1/miR168* homeostasis to be maintained in

every cell where the miRNA pathway is functioning [120]. Data also indicate that AGO1 is required for stem cell function and organ polarity [120].

The accumulation of *miR166* and *miR160* together with *miR393* could modulate morphological and hormone homeostasis by regulating transcripts of *HD-ZIPIII*, *ARF10*, and *TIR1* (Fig. 3). *HD-ZIPIII* was found to regulate the pattern of vasculature and the establishment and maintenance of abaxial-adaxial polarity in lateral organs [121]. ARFs regulate the expression of auxin-inducible genes, such as *GH3* and auxin/indole-3-acetic acid (Aux/IAA), by binding auxin-responsive promoters (ARPs) [122, 123]. In related studies, increased or decreased levels of *ARF10* altered *GH3-like* gene expression and led to severe developmental defects, *miR160*-resistant ARF plants were found to have dramatic developmental abnormalities, and *ARF10* was found to regulate floral organ identity and to be involved in seed germination [50, 122, 123]. The auxin receptor *TIR1* is an important SCF E3 ubiquitin ligase that functions in degrading Aux/IAA proteins in response to auxin [121] and then triggers adventitious root formation and lateral root development. *miR393*-mediated inhibition of *TIR1* would downregulate auxin signaling and seedling growth under biotic- and abiotic-stress conditions. Also, the upregulation of *miR156* and *miR319/159* could help the plant adapt to stress by modulating plant morphological characteristics via regulation of transcripts of *SBPs* and *MYBs/TCPs* transcription factors (Fig. 3). *SBP-LIKE* is involved in floral transition and regulation of flowering [125, 126]. Recent results indicate that overexpression of *miR156* affects phase transition from vegetative growth to reproductive growth by causing a rapid initiation of rosette leaves, a severe decrease in apical dominance, and a moderate delay in flowering [125–127]. *TCP* transcription factors direct the developmental processes determining leaf size, leaf form, and flower symmetry [127, 128]. Overexpression of *miR319*, which specifically downregulates *TCP* mRNAs, results in uneven leaf shape and delayed flowering [128]. Overexpression of *miR159a* specifically reduces *MYB* mRNA accumulation and results in male sterility, whereas plants that express *miR159*-resistant *MYB33* have upwardly curled leaves, reduced stature, and shortened petioles [128, 129]. Reyes and Chua [49] demonstrated how ABA induces the accumulation of *miR159* in association with the seed-specific transcription factor *ABI3*. In turn, *miR159* mediates cleavage of *MYB101* and *MYB33* transcripts, which encode positive regulators of ABA responses.

4 Conclusions and outlook

A complete understanding of the actions of small RNAs depends on the identification of the target genes. Identification of entire sets of miRNAs and siRNAs and their targets will lay the foundation that is needed to unravel the complex miRNA- and siRNA-mediated regulatory networks controlling development and other physiological processes. Given that miRNAs and siRNAs are crucial components of in gene regulatory networks, we believe that a complete understanding of the functions of miRNAs and siRNAs will greatly increase our understanding of plant tolerance to biotic and abiotic stresses.

Analysis of the DNA methylation profiles and the small RNA profiles will identify genes or regions that are regulated by miRNA/siRNA-mediated DNA methylation, which may contribute to epigenetic inheritance of stress effects. Also, the artificial microRNA (amiRNA)-mediated approach should have broad applicability for engineering multiple stress-responsive genes in crop plants.

Research Highlights

- Small RNAs are important regulators of gene expression in plants and animals.
- Plant small RNAs play a role in biotic and abiotic stress responses.

- The expression of many miRNAs is responsive to various stresses.
- Stress-responsive small RNAs are crucial components in gene regulatory networks.

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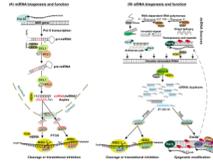


Fig. 1.

Biogenesis and function of miRNAs and siRNAs. (A) *MIR* genes are initially transcribed by Pol II into a single-stranded RNA that folds back to form a hairpin structure (also called pri-miRNA) thought to be stabilized by the RNA-binding protein DAWDLE (DDL) [138]. Splicing and further processing in nuclear dicing bodies involves the interactive functions of HYL1 and SE and of the cap-binding proteins (CBP) CBP20 and CBP80 [139, 140]. Pri-miRNAs and pre-miRNAs are generally thought to be processed from the free-end opposite to the loop by DCL1 to yield one or several phased miRNA/miRNA* duplexes. These are then methylated by HEN1 and transported to the cytoplasm by HST1. The miRNA guide strand is selected, incorporated, and stabilized in dedicated AGO1 protein. miRNA-guided AGO1-containing RISC directs mRNA cleavage or translation inhibition of the target transcript. miRNA-guided AGO1 functions are promoted by SQUINT (SQN) and HSP90 [22, 141]. (B) Various sources of dsRNA, its processing into siRNAs by one of four DCLs proteins assisted by dsRNA-binding proteins, HEN1-mediated siRNA stabilization, and selected strands of siRNA duplexes guide AGO-containing RISC to target RNAs for endonucleolytic cleavage and for translation repression, or these siRNAs can then guide AGO4 or AGO6 to function in RNA-directed DNA Methylation (RdDM) pathway involving Pol IV and Pol V. Cytosine methylation at these specific sites involves different effectors like the *de novo* methyltransferase DOMAINS REARRANGED METHYLTRANSFERASE 2 (DRM2); DEFECTIVE IN RNADIRECTED DNA METHYLATION 1 (DRD1), a member of the SWI2-SNF2 chromatin remodeling protein family; and DEFECTIVE IN MERISTEM SILENCING 3 (DMS3), a structural maintenance of chromosomes (SMC) protein.



Fig. 2.

Summary of stress-regulated small RNAs and their target families. Small RNAs are categorized based on the stress that they respond to. Grey box: abiotic stress, dark grey: biotic stress, green boxes: up-regulated small RNA, light green boxes: slightly up-regulated small RNA, and red boxes: down-regulated small RNA. At (*Arabidopsis thaliana*), Bd (*Brachypodium distachyon*), Bn (*Brassica napus*), Br (*Brassica rapa*), Gm (*Glycine max*), Mt (*Medicago truncatula*), Nt (*Nicotiana tabacum*), Os (*Oryza sativa*), Pt (*Populus trichocarpa*), Pta (*Pinus taeda*), Ptr (*Populus tremula*), Pp (*Physcomitrella patens*), Pv (*Phaseolus vulgaris*), Ta (*Triticum aestivum*), Zm (*Zea mays*). See the text for references.



Fig. 3. Regulatory network of stress-responsive miRNAs in *Arabidopsis*. A network is proposed that describes the molecular mechanisms underlying the response of *Arabidopsis* plants to different biotic and abiotic stresses. The network is based on the changes in expression profiles of miRNA and subsequent target transcripts in plants under stress. Green boxes: up-regulated RNAs; red boxes: down-regulated RNAs.

Table 1

Role of conserved plant miRNAs

Role	miRNA family	Target families/genes	Reference(s)
Auxin signaling	miR160	ARF10	[122, 123]
	miR164	NAC1	[130]
	miR167	ARF8	[122]
	miR390	ARF	[114]
	miR393	TIR1/F-box AFB	[15, 124]
Leaf development	miR159	MYB	[48, 127, 128]
	miR164	NAC1	[132]
	miR166	HD-ZIPIII	[131]
	miR172	AP2	[127]
Leaf polarity	miR319	TCP	[128]
	miR166	HD-ZIPIII	[121, 131]
	miR168	AGO1	[120]
Floral organ identity	miR390	ARF	[114]
	miR160	ARF10	[122, 123, 127, 128]
	miR164	NAC1	[132, 133]
Flowering time	miR172	AP2	[134]
	miR319	TCP	[127, 128]
	miR156	SBP	[125–127]
	miR159	MYB	[48, 127, 128]
Adaptive responses to stress	miR172	AP2	[127, 135]
	miR319	TCP	[127, 128]
	miR156	SBP	[37, 43, 44, 103]
	miR159	MYB	[16, 37, 43, 48, 49]
	miR160	ARF10	[37, 50, 100]
	miR167	ARF8	[37, 42, 43]
	miR168	AGO1	[37]
	miR169	NFY/MtHAP2-1	[37, 43, 52, 110, 136]
	miR171	SCL	[37, 43]
	miR319	TCP	[16, 37, 43]
	miR393	TIR1/F-box AFB	[15, 16, 37, 42, 43]
	miR395	APS/AST	[15, 16, 37]
	miR396	GRF	[16, 37]
	miR397	Laccases, Beta-6-tubulin	[15, 16, 37]
	miR398	CSD	[15, 19, 37, 43, 53, 72]
Regulation of miRNA	miR399	UBC24/PHO2	[36, 37, 75, 76]
	miR408	Plastocyanin	[16, 37, 44]
	miR162	DCL1	[137]
	miR168	AGO1	[120]
	miR403	AGO2	[114]

Role	miRNA family	Target families/genes	Reference(s)
Others	miR158	At1g64100	
	miR161	PPR	
	miR163	At1g66700, At1g66690	
	miR173	At3g28460	
	miR174	At1g17050	
	miR175	At5g18040, At3g43200, At1g51670	
	miR394	F-box	