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Review

Plant Small RNAs: Biogenesis, Mode of Action and Their Roles in Abiotic Stresses

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Abstract

Small RNAs (sRNAs) are 18-30 nt non-coding regulatory elements found in diverse organisms, which were initially identified as small double-stranded RNAs in *Caenorhabditis elegans*. With the development of new and improved technologies, sRNAs have also been identified and characterized in plant systems. Among them, micro RNAs (miRNAs) and small interfering RNAs (siRNAs) are found to be very important riboregulators in plants. Various types of sRNAs differ in their mode of biogenesis and in their function of gene regulation. sRNAs are involved in gene regulation at both transcriptional and post-transcriptional levels. They are known to regulate growth and development of plants. Furthermore, sRNAs especially plant miRNAs have been found to be involved in various stress responses, such as oxidative, mineral nutrient deficiency, dehydration, and even mechanical stimulus. Therefore, in the present review, we focus on the current understanding of biogenesis and regulatory mechanisms of plant sRNAs and their responses to various abiotic stresses.

Key words: plants, abiotic stress, small RNA, microRNA, siRNA

Introduction

Earlier science revolved around proteins, considering them as the sole regulatory molecules of the genome (*1*). Till then a lot of work was carried out to understand the regulatory mechanisms of eukaryotic gene expression. The first landmark in the field of gene silencing and small RNA was made in early 1990's.

Identification of sRNAs

In 1998, Fire *et al* (*2*) observed a small double-stranded RNA (dsRNA) in *Caenorhabditis elegans* acting as a regulator that switched off translation. Afterwards, with the initiation of sequencing projects and development of high-throughput deep sequencing methodologies, genes encoding small RNAs (sRNAs) have been identified. Interestingly, the genome of *C. elegans* was reported to contain 1,300 genes coding for functional non-coding RNA (ncRNA) transcripts (*3*). These genes were termed as ncRNA genes, which transcribed functional RNAs, although translation was not observed (*4*). During the Human Genome Project, it was found that only 1.06% of the human genome had the ability of encoding proteins. The non-coding sequences (~98%) comprises of introns and untranslated regions (UTRs) (27%), repetitive sequences (46%) and regulatory elements (25%) including ncRNA genes (*5*). With the sequencing of organisms

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in addition to *C. elegans* and *Homo sapiens*, it was generalized that as the complexity of organism increased, the protein-coding portion of genome decreased. From bacteria to humans, the percentage of the protein-coding sequences in genome decreased from 91% in *Mycobacterium tuberculosis*, 86% in *Escherichia coli*, 70% in *Saccharomyces cerevisiae*, 27% in *C. elegans*, 29% in *Arabidopsis thaliana*, 20% in *Drosophila melanogaster* to 1.4% in *H. sapiens* (*5-10*). Genomic DNA and its RNA counterparts regulate gene expression at transcriptional, post-transcriptional or translational levels (*1*). sRNAs have been identified as a component of genome in less evolved organisms. However, with the increase in complexity of organism levels, genome complexity increased, which might lead to the co-evolution of sRNAs and the associated ancient regulatory mechanisms of sRNAs. Thus it is speculated that with the increased complexity of organisms from less evolved to highly evolved eukaryotes, ncRNAs may undergo modifications. In higher eukaryotic organisms, ncRNAs might play a distinctive role in regulating the host systems at various regulatory levels, compared to that in lower organisms.

Classification of sRNAs

RNAs are classified into coding and non-coding RNAs (*1*) (**Figure 1**). ncRNAs have been variously classified depending upon their origin and functions (*11-13*) (**Table 1**). In *C. elegans*, ncRNA genes have been described as a repertoire consisting of transfer RNA (tRNA) genes, ribosomal RNA (rRNA) genes, trans-spliced leader RNA genes, microRNA (miRNA) genes, spliceosomal RNA genes and small nucleolar RNA genes. It was later demonstrated that other organisms do possess ncRNA genes (*3*). Among the various classified ncRNAs, sRNAs have been extensively studied.

sRNAs are a class of double-stranded RNAs with 20-30 nucleotides (nt) in length. They tend to target chromatin as well as transcripts, thus regulating both genome and transcriptome. But the term sRNA is rather a misnomer. This is because all known types of ncRNAs are recognized as small RNAs. In addition,

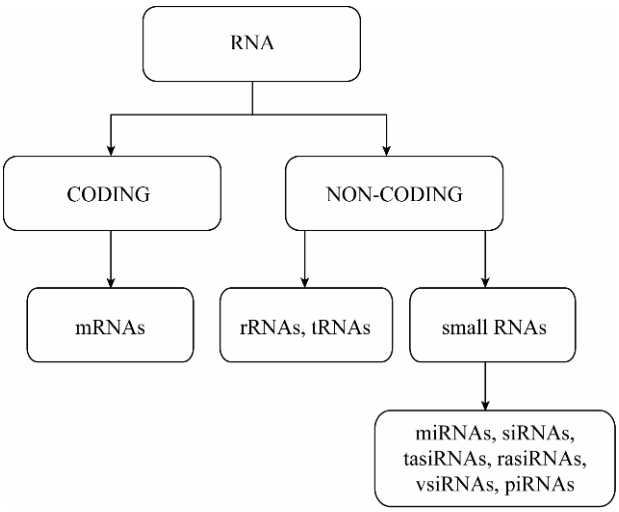

Figure 1 Types of RNAs. RNAs are classified into coding and non-coding RNAs. Coding-RNAs comprise of messenger RNAs (mRNAs). Non-coding RNAs are sub-divided into ribosomal RNAs (rRNAs), transfer RNAs (tRNAs) and small RNAs. Small RNAs constitute microRNAs (miRNAs), short interfering RNAs (siRNAs) and piwi interacting RNAs (piRNAs).

Table 1 Classification of eukaryotic ncRNAs

Basis of classification	Types	Ref.
Origin, properties and functions	1.DNA markers, playing roles in dosage compensation and imprinting: *Xist, roX, PAT-1*	*11*
	2.Gene regulators, affecting activity of genes: *DISC2, RNAI, RNA-OUT*	
	3.Abiotic stress signals, synthesized/processed in response to abiotic stress: *gadd7/adapt15, adapt33,G90*	
	4. Biotic stress signals, induced by biologically active compound: *His-1, CR20, GUT15*	
Predicted functions	1.Cellular debris ncRNA: RNAs with no specific function	*13*
	2.Housekeeping ncRNA: tRNA, rRNA, small nuclear RNA, small nucleolar RNA, signal recognition particle RNA	
	3.Regulatory ncRNA: miRNAs, siRNAs	
Role in RNA silencing	1. miRNA	*12*
	2. siRNA	

bacterial short regulatory RNAs are also designated by the same term. So, the unique feature distinguishing eukaryotic sRNAs from remaining known RNAs of genome is their small size (20-30 nt) and tendency to bind with Argonaute (AGO) family proteins (*14, 15*). AGO proteins are the sRNA effector proteins and the key components of RNA-induced silencing complex (RISC). These proteins are involved in directing mature sRNA to its target mRNA (*16*).

Though both sRNAs and protein-coding RNAs (mRNAs) possess variations, sRNAs can effectively regulate gene expression, gene splicing, nucleotide modifications and protein transport (*1*). The differences and similarities between sRNAs and mRNAs are presented in **Table 2**.

sRNA-mediated gene silencing was observed in eukaryotes long time back, but the mechanism behind it was not revealed then. As a result, sRNA-mediated silencing was named variously as RNA interference, co-suppression or quelling (*12*). Various types of sRNAs have been identified like microRNAs (miRNAs), small interfering RNAs (siRNAs), piwi interacting RNAs (piRNAs), small temporal RNAs (stRNAs), tiny non-coding RNAs (tncRNAs) and small modular RNAs (smRNAs). Among them, miRNAs and siRNAs have been characterized in plant as well as animal systems whereas piRNAs have been identified only in animals (*17, 18*). miRNAs are 20-22 nt in length and siRNAs are 21-24 nt long. Various types of siRNAs have been identified, including trans-acting siRNAs (Ta-siRNAs), repeat-associated siRNAs (Ra-siRNAs) and natural-antisense transcript-derived siRNAs (Nat-siRNAs) based on biogenesis and functions. The types that have been studied in most detail are Ta-siRNAs and Ra-siRNAs (*18*). A list of plant species in which sRNA studies have been carried out is given in Table S1. piRNAs have been specifically reported in animal germ cells, which are slightly bigger (26-31 nt) in size than sRNAs discussed previously. piRNAs are found to associate with Piwi domain of AGO family proteins (*14*).

Biogenesis of sRNAs

In plants, miRNAs are processed from single-stranded hairpin precursors ranging between 64-303 nt, while in animals, the size of miRNA precursors lies between 60-70 nt. This suggests an increased variability in the size of miRNA precursors in plants (*19, 20*). The miRNA biogenesis in plants is shown in **Figure 2A**. Genes encoding miRNAs in plants are annotated as MIR genes. Primary miRNAs (pri-miRNAs) are generated from MIR genes by the activity of RNA polymerase II (RNA pol II). The pri-miRNAs are processed

Table 2 Differences and similarities between sNAs and mRNAs

Property	Non-coding RNAs	Protein coding RNAs	Ref.
Length	20-30 nt (processed small RNAs) 64-303 nt (plant precursors) 60-70 nt (animal precursors)	Polynucleotides	*12*
Location of synthesis	Nucleus and cytoplasm	Nucleus and cytoplasm	*21*
RNA polymerase required	RNA polymerase II and IV	RNA polymerase II	*33*
Protein synthesis	No	Yes	*1*
Binding to Argonaute protein	Yes	No	*15*
Expression pattern	Mostly tissue- and developmental stage-specific expression	Only few with tissue- and developmental stage -specific expression	*17*
Energy consumption	Expressed without translation, requiring less energy	Translation, requiring relatively higher energy	*9*
Degradation rate	Less stable	More stable	*9*
Open reading frames	Absent	Present	*9*
Response to point mutations	Less sensitive	More sensitive	*9*
Effect of point mutations	More drastic effect	Less effect	*9*
Functions	Transcriptional and post-transcriptional gene silencing	Expression of genes	*1*
Identified types	miRNAs, siRNAs, tasiRNAs, rasiRNAs, vsiRNAs, piRNAs	mRNAs	*17*

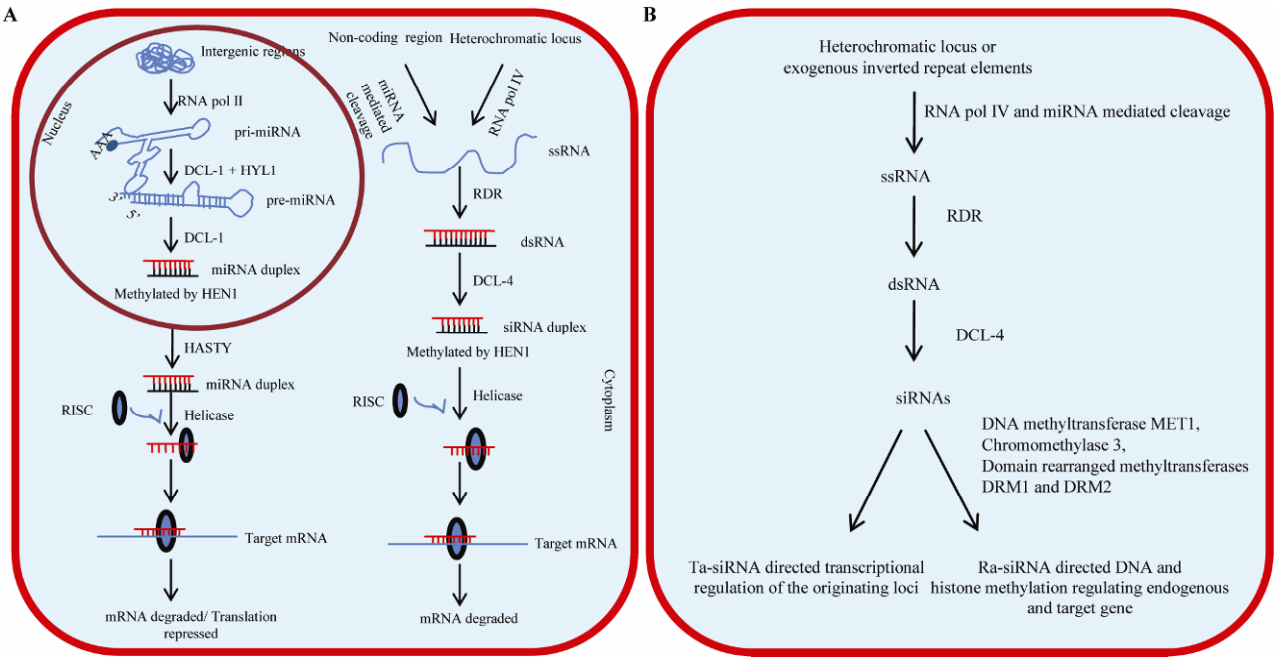

Figure 2 Diagrammatic representation of sRNA (miRNA and siRNA) biogenesis and sRNA-mediated transcriptional and post-transcriptional regulatory mechanisms. **A**. Biogenesis of miRNA and siRNA in plants. miRNAs are processed from intergenic regions of the genome. RNA pol II and Dicer-like 1 (DCL-1) in presence of protein Hyponastic Leaves 1 (HYL1) help form miRNA duplex, *i.e.*, miRNA-miRNA*. Duplex is stabilized due to methylation caused by Hua Enhancer 1 (HEN1). HST (HASTY) protein transfers miRNA duplex from nucleus to cytoplasm. Unknown helicases unwind the duplex making it accessible to RNA-induced silencing complex (RISC). Binding of miRNA to RISC directs the complex to target mRNA causing either its degradation or translation repression. The biogenesis of siRNAs begins depending upon the type of siRNA being synthesized. There are two kinds of siRNA precursors: non-coding regions for Ta-siRNAs and heterochromatic locus for Ra-siRNAs. Single-stranded precursors are processed for the respective siRNAs by miRNA-mediated cleavage or activity of RNA pol IV. dsRNAs are transcribed from ssRNA precursors by RNA-Dependent RNA polymerases (RDRs). DCL-4 slices dsRNA to form siRNA duplex that is methylated by HEN1. The siRNA duplex is untwined by a helicase and then binds to RISC that is targeted to its complementary mRNA. siRNA binds to its target mRNA and degrades the sequence. **B**. siRNA-mediated transcriptional gene regulation in plants. After the biogenesis of siRNAs as described in Panel A, siRNAs direct DNA methylation-responsive methyltransferases DNA methyltransferase MET1, CHROMOMETHYLASE3 (CMT3), domains rearranged methyltransferase 1 and 2 (DRM1/DRM2) to confer histone methylation that in turn transcriptionally regulates the expression of siRNA-originating loci in the case of Ta-siRNAs, and endogenous and target genes in the case of Ra-siRNAs.

inside the nucleus by ribonuclease III-like enzyme and Dicer-like 1 (DCL1) in association with Hyponastic Leaves 1 (HYL1) protein to produces mature miRNA duplex. The mature miRNA duplex contains two strands, namely miRNA and miRNA* that has 2 nt overhang at 3' end, compared to miRNA strand (*21*). The miRNA duplex is methylated by HEN1 (Hua Enhancer 1), a methyltransferase found in both plants and animals (*22*). The addition of methyl group at 2'-OH of the sugar residue to 3' nucleotide by HEN1 protects the miRNA from 3'-exonuclease degradation and 3'-uridylation (addition of short poly-U tail to unmethylated miRNAs that decreases its stability facilitating miRNA decay). The duplex is then transferred to the cytoplasm by a nuclear membrane protein known as HASTY (HST) (*18, 23-25*). Upon entry into cytoplasm, the duplex is acted upon by a helicase that is still unknown, which unwinds the miRNA-miRNA* duplex and exposes the mature miRNA to RISC. Mature miRNA thus binds with AGO protein-containing RISC and thereby directs RISC towards target mRNAs, leading to the cleavage or suppression of the translation of these mRNAs (*26, 27*).

siRNAs are closely related to miRNAs but they differ in terms of their origin, structure, associated effector protein and mode of action (*28, 29*). The differences between miRNAs and siRNAs are pre-

sented in **Table 3**. There are endogenous as well as exogenous precursors for siRNAs, *i.e.*, they are derived from the expressed products of host's own genome as well as directly from external viruses or transgene trigger. However, miRNAs have only endogenous, single-stranded precursors (*19, 20, 29*). The precursors for siRNAs are usually long and double-stranded (*12, 20*). The two types of siRNAs, Ta-siRNAs and Ra-siRNAs, differ in their precursors and certain steps of synthesis (**Table 4**). Ta-siRNAs are processed from ncRNA precursors, while Ra-siRNAs are generated from transposable and repetitive elements. The most distinguishing feature of Ta-siRNA biogenesis is the requirement of miRNA-dependent processing for generation of ssRNA precursor (*30-32*), while for Ra-siRNAs, a DNA-dependent RNA polymerase RNA pol IV transcribes ssRNA precursor from the heterochromatic locus (*33*). Later in both type of siRNAs, ssRNA precursors are duplicated to synthesize dsRNA precursors by RNA-dependent RNA polymerases (RDRPs). The rest of siRNA biogenesis pathway proceeds in the same way as miRNA (**Figure 2A**). Difference lies only in the type of enzymes and proteins involved.

The biogenesis of Ra-siRNAs, which is also known as heterochromatic siRNAs, has some peculiar features. It has been reported that RNA pol V generates non-coding transcripts from heterochromatic loci that direct AGO4-siRNA complex to the target mRNA. A study has recently shown that the production of RNA

Table 3 Differences between plant miRNAs and siRNAs

Property	miRNAs	siRNAs	Ref.
Definition	Regulators of endogenous genes	Defenders of genome integrity in response to foreign or invasive nucleic acids	*29*
Discovery time	1993	1999	*28*
Length	20-22 nt	21-24 nt	*18*
Precursors	Hairpin shaped ssRNAs	Long dsRNAs	*12*
Nature of precursors	Endogenous precursor gene of host's genome	Transposons, transgenes, repeat elements or viruses, *i.e.*, exogenous precursor	*29*
Mode of action	mRNA degradation, translational repression	DNA methylation, histone modification and mRNA degradation	*18*
Argonaute required	AGO1, AGO10	AGO1, AGO4, AGO6, AGO7	*14, 69*
Mechanism of gene regulation	Post-transcriptional only	Transcriptional as well as post-transcriptional	*18*
Complementarity with target sequences	Partially or fully complementary	Fully complementary	*29*
Functions	Cell development and cell differentiation, regulation of development processes, biotic and abiotic stress responses	Defense against transposons and viruses, stress adaptation	*17, 18, 92*

Table 4 Differences between eukaryotic Ta-siRNAs and Ra-siRNAs

Property	Ta-siRNAs	Ra-siRNAs	Ref.
Length	21 nt	24 nt	*29*
Precursor sequence	Non-coding RNA precursor, intron of a non-coding region	A heterochromatic locus	*30, 31*
Origin of dsRNA precursor	miRNA cleavage followed by activity of RDR6	RNA polymerase IV catalyzed transcription followed by activity of RDR2	*30, 31, 33, 68*
Dicer required	DCL-1, DCL-4	DCL-3	*12, 70*
Argonaute required	AGO1, AGO7	AGO4, AGO6	*35*
RNA polymerase V	No role	Transcribing heterochromatic loci to produce non-coding precursors, directing AGO4/siRNA to target mRNA	*36*
Regulatory function	mRNA degradation	DNA and histone modification	*32, 68, 70*
Source	Nematodes and plants	Plants and yeasts	*12, 66, 67*

pol V transcript is regulated by a chromatin remodeling protein defective in RNA-directed DNA methylation 1 (DRD1) and a structural maintenance of chromosomes (SMC) hinge-domain protein defective in meristem silencing 3 (DMS3) (*34, 35*). Interestingly, RNA pol II was shown most recently to demonstrate similar activity as RNA pol V (*36*).Various enzymes and proteins involved in miRNA and siRNA biogenesis are listed in Table S2.

Mode of action

Both miRNAs and siRNAs have been defined as riboregulators. They act on either RNA or DNA and regulate the expression of gene at transcriptional and post-transcriptional levels (*37-40*). miRNAs act post-transcriptionally through mRNA degradation or translational repression, whereas siRNAs function transcriptionally and post-transcriptionally by triggering DNA methylation, histone modification and mRNA degradation (**Figure 2B**) (*18, 37-42*). miRNAs are more important and abundant than the remaining sRNAs in the plant system. miRNA-mediated gene silencing is termed as heterosilencing because the genes that synthesize miRNAs and the genes regulated by miRNAs are different (*28*).

Gene regulation by sRNAs

sRNAs like miRNAs, Ta-siRNAs and Ra-siRNAs have different mechanisms of gene regulation. In the following section, we will discuss the regulatory mechanism of miRNAs and siRNAs in eukaryotes.

miRNA-mediated gene regulation

The gene regulation by miRNAs occurs in three different ways: (1) target mRNA degradation, (2) repression of translation and (3) miRNA-mediated mRNA decay (*43*). Among them, mRNA degradation and translation repression, are the most common ways of gene regulation. Complementarities between miRNA and the target mRNA determines in which way silencing is going to occur. When miRNA sequence and target sequence pair perfectly or almost perfectly, target mRNA is cleaved, while the imperfect pairing between miRNA and the target mRNA causes translation repression without cleaving mRNA (*28, 44*). As complementarities play a key role during mRNA degradation, the number of miRNA binding sites on target mRNA determines the rate of translation inhibition (*45*). The more the binding sites of miRNA on target mRNA, the more efficient the translation repression is (*12*). miRNAs have been reported to bind at 5'UTR, ORF and 3'UTR (*46, 47*). It has been shown that during protein synthesis miRNAs halt the movement of ribosomes along mRNA, thus repressing translation. However, this is not the universally accepted criteria, as certain miRNAs have perfect complementarities with mRNA but still inhibit translation irrespective of mRNA cleavage (*48-50*).

Various studies have evidenced a third and newer way of miRNA-mediated regulation. When miRNA shares partial or very little similarity with the target mRNA, miRNA decays the target mRNA instead of degrading or affecting translation of the target mRNA (*51-53*). The mechanism behind miRNA-mediated mRNA decay is that miRNA accelerates the removal of poly-(A) tail from the mRNA, making it unstable and finally leading to its decay (*54*). This mechanism has only been reported in animals till date. Furthermore, this mechanism of miRNA-mediated mRNA decay has been well illustrated in *D. melaogaster*. It has been reported that in S2 cells of *D. melanogaster*, P-body protein GW182 interacts with AGO1 and promotes the decay of target genes (*55*).

miRNAs have found regulatory role in various plants. As the time is lapsing by, more and more miRNAs are being reported. There has been a report revealing the identification of 180 miRNA loci from *Arabidopsis*, with their annotation and characterization into 80 miRNA families on the basis of expression and biogenesis criteria (*56-58*). With the identification of new plant traits, it becomes a challenge to pinpoint how they are regulated. miRNA-mediated control is likely one of the regulatory factors. It has been suggested that most of plant miRNAs are important for plant development (*59-61*). For example, reports have demonstrated the role of miR164 in organ initiation from meristematic tissues in *Arabidopsis*. miR164 down-regulates the responsible cup-shaped cotyledon (CUC) genes namely, CUC1, CUC2 and CUC3 post-transcriptionally (*62*). The role of miR164 also down-regulates the CUC genes during

normal flower development of *Arabidopsis* (*63*). miRNA-mediated mechanisms have also been shown in plant species other than *Arabidopsis*. In *Medicago truncatula*, transcription factor MtHAP2-1 has been reported to regulate differentiation of nodules. Further studies have documented that miR169 restricts the expression of MtHAP2-1 and facilitates the differentiation process (*64*).

miRNAs can be induced or repressed under stress conditions. However, miRNAs might be working in association with the constitutive defense mechanisms of plants to enhance their survival rate. Therefore, miRNAs may act as helping hand to reduce the work load of innate plant defense and regulatory systems (*56*).

siRNA-mediated gene regulation

Two types of siRNA-mediated gene regulation are known, which are governed by two different types of siRNAs.

Ta-siRNA-mediated regulation

Ta-siRNAs regulate gene expression at both transcriptional and post-transcriptional levels via target mRNA degradation and translational repression as miRNAs do. Ta-siRNA-mediated regulatory activity has been found only in plants and nematodes (*65-67*). Moreover, it has been reported that genes encoding Ta-siRNAs are not conserved among plant species. These studies suggest recent emergence of this type of sRNAs. Depending upon the complementarities between Ta-siRNAs and target mRNA, they cause cleavage of the target sequence (*31, 32*).

Ra-siRNA-mediated regulation

Ra-siRNAs are known to act primarily at transcriptional level in two ways, *i.e.*, DNA methylation and histone methylation. They are involved in DNA methylation and the methylation of lysine at ninth position of histone H3 (H3K9) causing systemic silencing (*68-70*). It has been reported that Ra-siRNA/AGO4 RISC directs DNA methyltransferases and H3K9 methyltransferases towards target sequence for the purpose of transcriptional gene silencing (*30, 70-72*).

The aforementioned sRNAs have been known to regulate functional expression of the associated genes by either up-regulation or down-regulation. Hence, sRNAs are involved in the regulation of various metabolic and physiological changes that occur under various environmental stress conditions.

sRNAs and abiotic stresses

Plants are exposed to various abiotic stresses such as salinity, drought, heat, heavy metals and nutrient deprivation during their life, which affect their growth and development at large scale. Moreover, primary stresses lead to secondary stresses like oxidative and thermal stress. These stresses induce similar and synergistic cellular damage to the plants by disturbing the ionic and osmotic homeostasis. Such disturbances can cause changes even at genetic level, disrupting the normal growth and leading to death under certain circumstances. These observations suggest the interconnection of the activities of various kinds of stresses (*73-75*).

Plants respond to these adverse conditions in various forms, broadly categorized as: (1) physiological responses, which could be stress-associated proteins and stress-associated metabolites; and (2) genetic responses mediated through epigenetic regulations. Physiological responses involve various proteins, transcription factors and metabolites (*76, 77*). Genetic responses mostly involve epigenetic changes, including RNA-directed DNA methylation, histone and DNA modifications, which play an important role in altering gene expression against abiotic stress (*78*). As described earlier in mode of action of miRNAs and siRNAs, these sRNAs play role in modifications of DNA, RNA and histone. Various groups have reported the presence of miRNAs among plant tissues under stresses. It has been reported that regulation of stress-related genes and miRNAs are somehow correlated (*74*). A vast study has already been carried out in this context in plant systems such as in *Arabidopsis thaliana*, *Oryza sativa*, *Zea mays* and *Triticum aestivum* (*48, 74, 79-81*).

miRNA and abiotic stress

For proper growth and reproduction, plants need an optimum quantity of every resource. Once the avail-

ability of any of these resources reaches above or below the threshold level, plant is abiotically stressed. Plants also experience abiotic stress through exposure of environmental factors like salinity, drought, heat, heavy metals, temperature and nutrient deprivation. Because of the sessile nature of plants, they have to cope with such environmental elements. Many genes encoding both transcription factors and important detoxifying enzymes have been identified. Currently, various miRNAs in several plant species like *Arabidopsis, Oryza*, *Nicotiana*, *Z. mays*, *Sorghum*, *Populus*, *Gossypium*, *Brassica*, *Vitis*, *Physcomitrella* and *Chrysanthemum* have been discovered (*82-94*). The majority of miRNA target genes are found to encode various transcriptional factors or important functional enzymes and to play important roles in plant development and in response to various abiotic stresses.

Specific miRNAs have also been discovered with important roles in protecting the plants against abiotic stresses (*74, 95*). As mentioned by Phillip Zamore, "It would be a cruel joke that nature has played on scientists if the miRNA sequences had been conserved in evolution but with no function" (*96*). Exposure to different kinds of abiotic stresses may lead to similar responses in plants. Also, different kinds of stresses have been found to trigger responses through induction of similar types of miRNAs. This suggests that plants share common pathways that are involved in different abiotic stress responses. The identified miRNAs are either up-regulated or down-regulated upon stress treatments that have an impact on plant growth and developmental processes. Here, the role of specific miRNAs in different abiotic stresses has been discussed below and compiled in Table S3.

miRNAs regulating stress caused by light

Plant growth is highly flexible because of its dependence on the surrounding environmental conditions. Of the various environmental factors, light acts as one of the essential elements and can be stress to plant like other kind of stresses (*97*). For example, exposure of radiations above the saturation point of photosynthesis causes high light stress effects (*98*). Interestingly, ~20% of expression in rice and *Arabidopsis* genome is regulated by white light. Both plants have possessed light-mediated regulation of genes, transcription factors and certain metabolic pathways (*99*). Among the various fractions of white light, UV-B fraction is considered as the harmful and deteriorating radiation which can affect plants, directly and indirectly. Although a minor proportion of white light, UV-B radiation has the capacity to cause molecular damage at the level of DNA, RNA and proteins and introduces alterations in the morphology and physiology of plants (*100*).

Based upon statistical algorithms and computational approaches, miRNAs involved in UV-B radiation stress have been reported. UV-B responsive miRNAs were predicted on the criteria that miRNAs should possess same array of proximal promoter motifs as the UV-B responsive protein-encoding genes and the inferred expression of miRNAs should be negatively correlated with the expression of target genes. It has been indicated that 21 miRNAs from 11 miRNA families are up-regulated during such stress. The reported UV-B responsive miRNA families are miR156/157, miR159/319, miR160, miR165/166, miR167, miR169, miR170/171, miR172, miR393, miR398 and miR401. These miRNAs, except for miR393, miR398 and miR401, have been found to target genes encoding transcription factors that subsequently affected the expression of related genes (*101*).

miRNAs regulating water stress

Loss of water as well as excess of water can act as stress for plants. The loss of water or drought stress induces morphological and physiological changes in plants. At the same time submergence or water excess causes anaerobic conditions around plants (*102, 103*). Plants regulate the expression of various genes at transcriptional and post-transcriptional level in response to such stress. They undergo various morphological, physiological and metabolic changes to adapt against water stress. Recently, genome profiling of drought stressed rice has been carried out at various developmental stages to reveal drought-responsive miRNAs. It has led to the identification of 30 miRNA families, which was either up-regulated or down-regulated significantly during drought. Detailed analysis has revealed that eight miRNAs from 30

families were up-regulated (miR395, miR474, miR845, miR851, miR854, miR901, miR903 and miR1125) and 11 miRNAs were down-regulated (miR170, miR172, miR397, miR408, miR529, miR896, miR1030, miR1035, miR1050, miR1088 and miR1126). Prediction and validation of target genes corresponding to these miRNAs and study of their regulation at the level of transcription factors have evidenced the role of these miRNAs in drought tolerance (*79*).

In maize, 39 miRNAs have been identified with altered expression under submergence stress. Among them, expression of 19 miRNAs was up-regulated during the early stages (0-12 h) of submergence, which recovered to normal levels during later stages. However, the expression of 12 miRNAs was down-regulated during the initial stages and up-regulated after 24 h of submergence. Interestingly, seven of these 39 miRNAs were dramatically induced between 24 h and 36 h of post-submergence. These miRNAs targeted genes that actively participate in eliminating reactive oxygen species (ROS) and aldehyde groups. Also, target genes possess a *cis*-acting element that is essential to cope with anaerobic conditions. The predicted targets of these miRNAs were classified into three categories. The first category includes various transcription factors involved in plant development and organ formation. For example, ZAG1, an agamous-like gene, was detected as a target of *Z. mays* miR159 (zma-miR159). In addition, HD-ZIP is a target for zma-miR166 and scare crow-like family (SCL) is a target for zma-miR171. The second category includes several targets of miRNAs that are involved in phytohormone cascade such as GAMyb and auxin responsive factors (ARF12, ARF17 and ARF25). The third category includes targets encoding the proteins involved in physiological processes. The predicted targets of submergence stress responsive miRNAs are involved in carbohydrate and energy metabolism, including starch synthase, invertase, malic enzyme and ATPase, as well as in elimination of ROS and acetaldehyde (ALDH). These findings have highlighted the complexity of adaptive plant responses. These adaptation strategies are helpful for survival of maize seedlings under submergence conditions (*103*).

miRNAs regulating sulfate stress

miR395 has been shown to be involved in the regulation of sulfur homeostasis (*104*). Sulfur is taken up by plants in the form of inorganic sulfate (*105*). Sulfur deficiency has resulted in some physiological changes that suspended sulfate assimilation. miR395 has two targets. The first target is ATP sulfurylase (APS1, APS3 and APS4) enzyme that catalyzes the first step of sulfur assimilation pathway (*95*). Whenever there was a sulfate starvation, miR395 expression was up-regulated. This has resulted in the down-regulation of APS transcript and consequently retarded the sulfate assimilation (*106*). The second target of miR395 is *AST68*, an *Arabidopsi*s sulphate transporter that is involved in the translocation of sulfate from roots to shoots (*107, 108*). This finding is interesting for miRNA-mediated gene regulation because miR395 appears to regulate two different groups of genes that function in the same metabolic pathway.

miRNAs regulating phosphate starvation

Phosphate is the key structural component of nucleic acids and membranes. It is also involved in many biological functions and is an active player in ATP-driven energy transfer reactions (*109*). The role of miRNAs in phosphate homeostasis has been studied (*104*). *Arabidopsis* miR399 has been found to target two genes, *i.e.*, a phosphate transporter (PHO2) and a putative ubiquitin conjugating enzyme (UBC24) involved in protein degradation (*74, 95*). Both targeted proteins are involved in maintaining the inorganic phosphate (Pi) homeostasis. miR399 regulates *UBC24* expression through RISC-mediated cleavage and more likely through translational repression (*110, 111*). Under Pi-sufficient conditions, expression of miR399 was suppressed, while expression of *UBC24* or *PHO2* was elevated. These were presumed to participate in an ubiquitin pathway that negatively regulated the expression of Pi transporters. Thus, hormonal signaling regulates the root growth to prevent the overloading of Pi. However, limitation of Pi has resulted in the up-regulation of miR399, which in turn down-regulated the expression of *UBC24* or *PHO2*. This down-regulation has been observed for increased

repression of Pi transporter genes and altered root growth to architect the maximization of Pi uptake, which ultimately enables the plant to cope up with the Pi deficiency (*111-114*). This is further supported by the evidence that plants with *pho2* mutation or miR399 overexpression showed a significant increase in a Pi transporter (*PHT1*) in roots (*114*). The mechanism by which *UBC24* influences Pi homeostasis remains unclear. It has been suggested that *UBC24* regulates the expression of a transcription factor that could be involved in Pi homeostasis (*115*).

MYB transcription factor and phosphate starvation response 1 (PHR1) are expressed in response to Pi starvation and hence, are involved in miR399 expression., PHR1 is involved in positive regulation of Pi-responsive genes by binding to GNATATNC *cis*-element (*115-117*), which is found upstream of all known miR399 genes in *Arabidopsis*. A significant decline in miR399 induction was demonstrated in *phr1* mutants under Pi stress (*111, 115*).

The shoots of miR399-overexpressing lines have been found to contain higher Pi than roots compared to wild-type plants. Chiou *et al* proposed that remobilization of Pi from shoots to roots may also be a factor in accumulation of Pi (*112*). It has also been shown that expression of both *UBC24* and miR399 is localized in the vascular cylinder of the root subjected to Pi starvation and such localization of *UBC24* and miR399 has a systemic effect on Pi translocation between roots/shoots and Pi mobilization within leaves (*114*). This was further evidenced by the grafting experiments showing accumulation of miR399 in the roots of Pi starved plants and a unidirectional flow of miR399 from the shoot to the root (*118*). In conclusion, Pi starvation induces the production of miR399 in shoots, which then shuttles to the roots and alters the expression of *UBC24*.

miRNAs regulating copper concentration and related stress conditions

miR398 has also been found to regulate the concentration of copper ions in the plant cell. Copper is an essential plant micronutrient involved in photosynthesis, oxidative stress response, *etc* (*109*). Copper starvation induces miR398 expression, which further suppresses the translation of *CSD1* and *CSD2* into Cu-SOD proteins (*119*). *CSD1* and *CSD2* genes are induced under oxidative stress in order to prevent superoxide radicals toxicity (*120*). Furthermore, support to this finding has been provided by the phenotypes of *Arabidopsis* plants expressing a miR398-resistant *CSD2* (*mCSD2*) mutant. Plants possessing *mCSD2* have demonstrated greater tolerance to conditions inducing oxidative stress as compared to wild-type plants (*121*). Reduction of Cu-SOD proteins increases the availability of copper for other biological processes (*109*). It suggests that miR398 is a part of regulatory network that controls the copper ion concentration in plants.

miRNAs regulating cold and related stress conditions

Cold stress is one of the most severe abiotic stresses. It adversely affects plant growth and development. Cold stress includes chilling (below 20ºC) and freezing conditions (below 0ºC). The chilling inhibits water uptake, while freezing induces cellular dehydration, thus causing an osmotic stress and hyperaccumulation of ROS as secondary effects (*122*). Plants are often acclimatized to cold conditions but certain plants are sensitive to these chilling and freezing conditions. Various genes and transcription factors are known to play an active role to deal with cold stress (*123-127*). Post-transcriptional regulations are considered more critical for cold tolerance (*123*). Among the known post-transcriptional mechanisms, sRNA-mediated regulation has been found to play an important role. From the *Arabidopsis* database Genevestigator, miRNA target genes responsible for the growth and developmental regulation of the plants have been ruled out from their possibly responses to cold stress. Microarray data has reported the involvement of certain miRNAs against cold stress.

From *Arabidopsis*, sRNA library was constructed to identify sRNAs involved in cold, dehydration and salt stress. This study has come out with the identification of two previously known miRNAs miR171b and miR319c, 24 novel miRNAs from 15 new families and 102 novel endogenous siRNAs. From the identified miRNAs, miR393, miR397b and miR402 were up-regulated during cold, dehydration and salt stress whereas miR389a.1 was down-regulated.

miR319c was found to be specifically up-regulated during cold stress (*74*). Recently, microarray based profiling of cold responsive miRNAs has also been carried out from rice (*127*). Most of the identified miRNAs were down-regulated during cold stress. miRNAs from miR167 and miR319 families were down-regulated, while those from miR171 were reported for variable expression profiles (*127*).

miRNAs regulating salt stress

It has been found that genes encoding laccase-like proteins (LAC) and a regulatory subunit of casein kinase (CKB3) are down-regulated by salt stress in *Arabidopsis* (*128*). This down-regulation was caused by salt stress-induced transcriptional up-regulation of miR397, which has directed the cleavage of *LAC* and *CKB3* transcripts. Interestingly, overexpression of miR397 in transgenic *Arabidopsis* enhanced *LAC* and *CKB3* transcript cleavage as well as the plant tolerance to salt stress. These results have demonstrated that down-regulation of *LAC* and *CKB3* transcripts guided by miR397 is essential for salt tolerance. Hence, manipulation of the expression of such miRNAs can be an effective new approach for improving the plant salt tolerance (*128*).

In rice, miRNAs from miR169 family have been reported as salt responsive. miR169g and miR169n (o) were significantly induced during salt stress, which caused cleavage of a CCAAT-box binding transcription factor carrying NF-YA gene, Os03g29760. In addition, study in *Arabidopsis* also indicated that one member of miR169 family was significantly induced upon salt treatments. Thus, miR169 was described as salt responsive miRNA family (*129*).

Salt responsive miRNAs have been identified from maize roots (*80*). miRNA microarray hybridization has lead to the identification of 98 salt responsive miRNAs from 27 plant miRNA families. These miRNAs showed differential expression during salt stress. While 18 miRNAs were expressed in maize salt tolerant species, 25 miRNAs showed delayed expression in maize salt sensitive species (*80*).

Most of the miRNAs responsive to salt stress directly regulate transcription factors. From *Z. mays*, miR159a/b, miR164a/b/c/ d and miR1661/m have been cloned that target transcription factors Myb, NAC1 and homeodomain-leucine zipper protein (HD-ZIP) (*80*). Other salt responsive transcription factors targeted by miRNAs included MADS-box proteins and zinc-finger proteins. Further experimentation has lead to the cloning of miRNAs belonging to miR474, miR395 and miR396 families from *Z. mays*. miR474 and miR395 were reported to target negative regulators of salt tolerance. They were up-regulated during salt stress, causing suppression of the respective factors. On the contrary, miR396 was reported to down-regulate in the presence of salt stress.

miRNAs regulating mechanical stress

Various mechanical stresses involving wind, water, or any other entity imposing physical forces upon the plant body have been found to down- or up-regulate certain miRNAs. A comparative analysis of miRNA expression was performed in *Populus trichocarpa* subjected to mechanical stress via bending the plant stem in an arch for 4 d (*88*). The expression of miR156, miR162, miR164, miR475, miR480 and miR481 was found to be down-regulated whereas miR408 was up-regulated in the xylem tissue of mechanically stressed plants as compared to the un-stressed control (*88*). Further studies on mutants overexpressing specific miRNAs or miRNA-resistant target sequences would aid in determining the role of these miRNAs.

miRNAs regulating hypoxia

Hypoxia is a stressful state caused by loss of oxygen. Various studies have been carried out to elucidate the mechanism behind plant response against hypoxia. Plants possess various hypoxia responsive transcription factors that possibly trigger anaerobic responsive genes. However, the exact mechanism is still not known. Recently, a study has indicated the role of miRNAs under such anaerobic conditions (*130*) by analyzing around 1,900 transcription factors and 180 miRNA primary transcripts in stressed *Arabidopsis*. It was found that simultaneous interaction of various transcription factors possibly regulates the hypoxia induced genes, although one miRNA (miR391) analyzed showed minor activity (*130*). Another study has

documented the role of miRNAs and siRNAs in assisting plant to survive under anaerobic conditions (*131*). Altered expression was observed for 46 miRNAs belonging to 19 miRNA families and three ta-siRNA families. miR156, miR157, miR159, miR172, miR391, miR775 and tasiRNA289 were reported as hypoxia responsive sRNAs (*131*).

miRNAs regulating ABA, dehydration and other environmental abiotic stresses

In *Arabidopsis*, miR417 was regulated by abscisic acid (ABA), dehydration and salt stress (*132*). Expression of miR417 was detected in all major tissues of *Arabidopsis*, which was influenced by multiple abiotic factors. High salt stress was shown to moderately decrease transcript levels of miR417, while ABA treatment and dehydration stress resulted in initial up-regulation followed by down-regulation of miR417. *Arabidopsis* seeds overexpressing miR417, when subjected to salt and ABA treatment, showed decreased rate of seed germination as compared to wild-type plants. The survival rate of the seedlings was also negatively affected. However, gene(s) targeted by miR417 still remains unknown (*132*).

Another miRNA found to be up-regulated by various abiotic stresses like ABA, dehydration, cold and NaCl is miR393. miR393 has been reported to regulate the expression of mRNAs encoding the F-box auxin receptor and transport inhibitor response 1 (TIR1) (*94, 133*). TIR1 in turn targeted AUX/IAA proteins for proteolysis by SCF-E3 ubiquitin ligases in an auxin-dependent manner. These proteins are known to be necessary for various auxin-induced growth and developmental processes (*134, 135*). These observations have documented that different abiotic stresses lead to increased TIR1 mRNA degradation or translational repression via miR393 accumulation, which negatively impacts auxin signaling and seedling growth (*74*).

Other sRNAs and abiotic stresses

Besides miRNAs, other sRNA molecules have also been proposed to function in stress adaptation (**Table 5**). The best characterized siRNA involved in adaptation against abiotic stress is nat-siRNA. It was first observed in salt stressed *Arabidopsis* and was involved in the regulation of proline metabolism.

Nat-siRNA was originally shown to match the overlapping region between the 3' end of pyrroline-5-carboxylate dehydrogenase (*P5CDH*) ORF and the 3' UTR of an unknown gene on the opposite strand, which was designated *SRO5* (*74*). SRO5 expression was induced under salt stress *SRO5* transcript complements with *P5CDH* transcript to form a

Table 5 Roles of other sRNAs in abiotic stresses

siRNA	Plant species	Abiotic stresses regulated	Target genes	Corresponding Protein annotations	Ref.
tasiR289	*Arabidopsis*	Hypoxia stress	At1g15940 At1g51670 At4g29770 At5g18040	Cysteine domain containing proteinases	*131*
SRO5-P5CDH nat-siRNA	*Arabidopsis*	Salt stress	At5g62530 At5g62520	P5CDH SRO5	*136*
02061_0636_3054.1 siRNA	Wheat	Heat, NaCl and dehydration stresses	-		*81*
005047_0654_1904.1 siRNA	Wheat	Cold, Heat, NaCl and dehydration stresses	- -		*81*
080621_1340_0098.1 siRNA	Wheat	Cold and Heat stresses	- -		*81*
007927_0100_2975.1 siRNA	Wheat	Cold, NaCl and dehydration stresses	-		*81*
CDT1-siRNA	*Craterostigma plantagineum*	Dehydration and ABA stresses	Y11822		*137*

dsRNA, which acts as precursor for a 24-nt nat-siRNA and mediates the cleavage of *P5CDH* transcripts. A 21-nt nat-siRNA was derived from the cleavage products, which mediates the cleavage of more *P5CDH* transcripts. The down-regulation of *P5CDH* reduced the proline degradation, and thereby enhanced proline accumulation. A high level of proline is a positive factor for salt tolerance. The accumulation of proline aids in stress tolerance by ROS scavenging and possibly through osmoprotection (*136*). A ta-siRNA289 has been reported as hypoxia responsive sRNA from *Arabidopsis*, which targets the genes coding for proteinases containing cysteine domain (*132*). The *Craterostigma* desiccation tolerant (*CDT-1*), which is a dehydration-related ABA-inducible gene from the well-known resurrection plant *Craterostigma plantagineum*, has been reported to direct the synthesis of an endogenous siRNA. This siRNA has significantly increased the dehydration tolerance of *Craterostigma* (*137*).

In the case of wheat, northern blot analysis has revealed that the expression of four siRNA changed greatly in the seedlings under various stress treatments like cold (4°C for 2 h), heat (40°C for 2 h), NaCl (200 mM) and dehydration stress (*81*). Interestingly, both siRNA 002061_0636_3054.1 and siRNA 005047_0654_1904.1 were down-regulated by heat, NaCl and dehydration stress, while the latter was also up-regulated by cold stress. In addition, siRNA 080621_1340_ 0098.1 was up-regulated by cold and down- regulated by heat but not by NaCl and dehydration stress, while siRNA 007927_0100_2975.1 was down-regulated by cold, NaCl and dehydration stress but not by heat stress (*81*). The genes targeted by these siRNAs could play roles in regulating stress responses in wheat. However, the mechanisms of these sRNAs in regulating gene expressions and the biological function of target genes have not been explored yet.

Conclusion

Expanding human population is increasingly stressing agro-ecosystems that result in constant subjection of plants to various abiotic stresses like salinity, drought, dehydration, heat, heavy metal, *etc*. Abiotic stress has always been taken as the biggest hurdle in the growth and development of plants. It has been found that various sRNAs work within the plants in response to such adverse conditions. Researches on sRNAs have emerged recently and within no time a lot of studies regarding their types, biogenesis, targets and functions have been conducted. But still the information is not enough to label them a well characterized class of ncRNAs. The work of various groups has led to the classification of sRNAs into mainly three categories, miRNAs, siRNAs and piRNAs, while many new types of sRNAs are under exploration. These three types of sRNAs differ from one another but at the same time, they collaborate in their mode of action. Their collaborative response has well suggested that these different sRNAs are actually interconnected at some points. In plants, both miRNAs and siRNAs are present. They act collectively as well as individually to help the plants with their maintenance, homeostasis and survival under adverse conditions. But still, functions of most sRNAs are currently unknown, and there is a large gap between the identification of sRNA genes and the verification of their functions. Presently, substantial researches are being conducted to find the role of miRNAs and siRNAs in abiotic stresses. New functions of miRNAs are being discovered in regulating gene expression in response to these stresses. For example, UV-B stress is very important because of its impact on depletion of ozone layer. A number of miRNAs induced or suppressed by UV-B stress in *Populus* are known but the regulatory role of these stress responsive miRNAs and their target genes is still unknown. Only few plants have been explored and much work is still ongoing in this regard. If the breadth of regulation by sRNAs is as predicted, they may be used as a promising tool to improve plant yields, quality, or resistance to various environmental stresses. Discovery of more sRNAs in plant system will provide researchers with an opportunity to manipulate these sRNAs in favor of plant growth and development. Hence, it will be very appropriate to call sRNA “an efficient molecule of the millennium”.

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Supplementary Material

Tables S1-S3

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