

The Attributes of RNA Interference in Relation to Plant Abiotic Stress Tolerance

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Abstract

Micro RNAs are small non coding RNA molecule that plays a vital role in post transcriptional gene regulation by either translational repression or by inducing mRNA cleavage. These small non coding RNAs have emerged as one of the master regulators of plant growth and development. Recent studies have revealed their role in abiotic stress responses. Expression level of several miRNA changes when exposed to drought, salinity, temperature variations and oxidative environment resulting in modulation of the expression of target genes that are associated with stress response. This review aims to focus on the regulatory role of plant micro RNAs during abiotic stress.

Keywords: RNAi; Abiotic stress; siRNA; miRNAs; Transcriptional repressor

Abbreviations

Ath: *Arabidopsis thaliana*; Bdi: *Brachypodium distachyon*; Hvu: *Hordeum vulgare*; Mtr: *Medicago truncatula*; Pvu: *Phaseolus vulgaris*; Peu: *Populus euphratica*; Ptc: *Populus trichocarpa*; Ttu: *Triticum turgidum*; Osa: *Oryza sativa*; Vun: *Vigna unguiculata*; Zma: *Zea mays*; UK: Unknown; *: Upregulated; #: Downregulated.

Introduction

RNA interference has become a new paradigm for understanding gene regulation. It is a highly conserved process which functions in a very sequence specific manner and induce gene silencing. The mechanism of action involves depletion and subsequent degradation of targeted mRNA by introducing double stranded RNA thereby inhibiting the expression of corresponding gene specifically and efficiently. RNAi is a very common phenomenon which was initially discovered in plants and then in nematode, flies, zebra fish and mammalian cells. It has emerged as an indispensable tool for loss of gene function studies across the eukaryotic world. There are several classes of small non coding RNAs that can regulate gene by targeting transcripts in the cytoplasm and repressing their translation. In plants, based on their biogenesis, functional categorization and the structure of genomic loci from which they are transcribed small regulatory RNAs can be classified into two major classes. The micro RNAs (miRNA) and the small interfering RNAs (siRNA). The main distinguishing features between miRNAs and siRNAs is the number of short RNA molecules generated from one loci. In case of miRNAs only one miRNA duplex is produced from one miRNA loci whereas many siRNA duplexes are generated from one siRNA loci. Another distinct character that differentiates siRNAs from miRNAs is that they are generated from long double stranded RNA molecules by the action of RNA dependant RNA polymerase. These small non coding RNAs have been found to affect various biological processes such as leaf morphogenesis, root development, and floral organ identity. Their roles in response to different abiotic and biotic stresses have also been studied by several workers [1]. In this article we have highlighted the

role of micro RNAs as an important gene expression regulator during plant abiotic stresses.

Plant Micro RNA Biogenesis and mechanism of its action

Micro RNAs are 20-24 nucleotide long non coding RNAs encoded by miRNA genes that are transcribed into primary miRNAs by RNA polymerase II which in turn is converted into precursor miRNA, a self-complementary stem loop secondary structure that often exhibit an imperfect double stranded character [2]. These precursor miRNA gives rise to the mature miRNA duplex by the action of dicer like (DCL) protein. The duplex thus formed associate itself with the ARGONAUTE (AGO) protein where a single strand of the duplex gets degraded [3]. Mostly this single stranded miRNA guide the AGO protein to recognize its target mRNA based on their near perfect complementarity, thus forming RNA induced silencing complex (RISC), subsequently cleaving or degrading its target mRNA. In another study it was observed that the miRNA repress the gene expression by inhibiting the progression of translational apparatus along the template mRNA thereby blocking protein synthesis [4].

Micro RNAs: Attributes in Plant Abiotic Stress responses

Plants combat environmental stresses by activating several gene regulatory pathways. Plant exposed to abiotic stress causes over or under expression of certain miRNA and might even lead to the synthesis of new miRNAs to withstand stress [1]. Several stress regulated miRNAs have been identified in different plants when subjected to various abiotic stresses such as drought, salinity, temperature fluctuation and oxidative environment. Studies with different model plants have revealed the role of these miRNAs in response to abiotic stress. In *Arabidopsis*, miRNAs such as miR396, miR168, miR167, miR165, miR319, miR159, miR394, miR156, miR393, miR171, miR158, and miR169 were identified as drought-responsive. A consistent upregulation of miRNA miR393, miR319 and miR397 has been shown in *Arabidopsis* in response to drought stress [5]. Genome wide analysis of micro RNAs were carried out in drought stressed rice plants belonging to different developmental stages using a microarray platform. It was observed 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 while another 14 miRNAs which include miR159, miR169, miR171, miR319, miR395, miR474, miR845, miR851, miR854, miR896, miR901, miR903, miR1026, and miR1125 were upregulated in response to drought stress [6]. Salinity is another major threat in agricultural agronomy. A huge number of salt regulated micro RNAs have been identified that plays either a direct or indirect role in salt stress alleviation. Several differentially regulated miRNAs have been identified in the salt stressed tissue of Arabidopsis. Salt stress causes upregulation of miR156, miR158, miR159, miR165, miR167, miR168, miR169, miR171, miR319, miR393, miR394, miR396, and miR397 while miR398 was found to be downregulated [7]. Results of microarray experiments used to study micro RNA profile of salt tolerant and salt sensitive maize variety indicates that the member of the miR156, miR164, miR167, and miR396 families were downregulated, while miR162, miR168, miR395, and miR474 families were upregulated in salt-shocked maize roots [8]. Up-regulations of miRS1, miR159.2 in *Phaseolous vulgaris* and miR530a, miR1445, miR1446a-e, miR1447, miR1711n in *Populus trichocarpa* were observed [9,10]. Temperature fluctuation causes plants to reprogram their gene expression profile to adjust with such dramatic shifts in temperature. During cold stress, comparative analysis of miRNA expression profiles of different species such as Arabidopsis, Brachypodium and poplar have been studied. MiR397 and miR169 were upregulated constitutively in all the three species whereas miR172 was only found to be upregulated in Arabidopsis and Brachypodium but not in poplar [11]. Again miR168 levels were upregulated in Arabidopsis and poplar but downregulated in rice [6,11]. Along with these cold stress responsive miRNAs, heat responsive miRNAs have also been identified in poplar and wheat. miR156, miR160 and miR162 were upregulated in wheat when the plant was exposed to heat stress while miR170 was downregulated in poplar in response to heat stress [13]. Anaerobic or hypoxic condition induces massive changes in the transcriptome profile of the plant. Several miRNAs from different species such as maize, rice, Arabidopsis and poplar were reported to be differentially regulated in

response to hypoxia. The abundances of 19miRNAs families significantly changed in response to hypoxia in Arabidopsis when exposed to hypoxic conditions [14].

Micro RNAs mostly target multiple genes belonging to the same families in plants. Many reports derived from previous studies reveal that miRNAs under specific condition can regulate the expression of specific target genes (Figure 1) [15]. In a study with maize plant it was observed that the salt responsive miRNAs mostly targets transcription factors those are involved in plant development, organ formation and stress regulation. The transcription factors Myb, NAC1 and homeodomain-leucine zipper protein were predicted as the targets of zma-miR159a/b, zma-miR164a/b/c/d and zma-miR166l/m, respectively. A similar kind of result was observed in Arabidopsis and rice [16]. In addition to the transcription factors, miRNAs also target genes that encode protein involved in various physiological and metabolic processes. During drought stress miR393 was found to be consistently upregulated in many plants such as Arabidopsis, Medicago, common bean, and rice that target genes encoding the TIR1 (Transport Inhibitor Response Protein 1)/AFB (Auxin Signaling F-box Protein) family of F-box auxin receptors [17]. Deep sequencing data showed that the expression of miR1425 was suppressed during cold stress in rice panicle. This miR1425 targets a set of gene encoding Pentatricopeptide repeat (PPR) proteins that are basically RNA binding proteins involved in post transcriptional processes such as RNA splicing, RNA editing and RNA stability [18]. Superoxide in plants that causes oxidative damage is converted into less toxic hydrogen peroxide and molecular oxygen by the enzyme superoxide dismutase (SOD). This is one of the most important enzymes involved in the ROS scavenging system. Cu-Zn SODs are encoded by CSD1, CSD2 and CSD3 in Arabidopsis and miR398 was predicted to target these genes. It was observed that miR398 is downregulated under oxidative stress which is accompanied with accumulation of CSD1 and CSD2 transcripts. The accumulation of these transcripts occurs due to the inhibition of miR398 directed cleavage [19] (Figure 1).

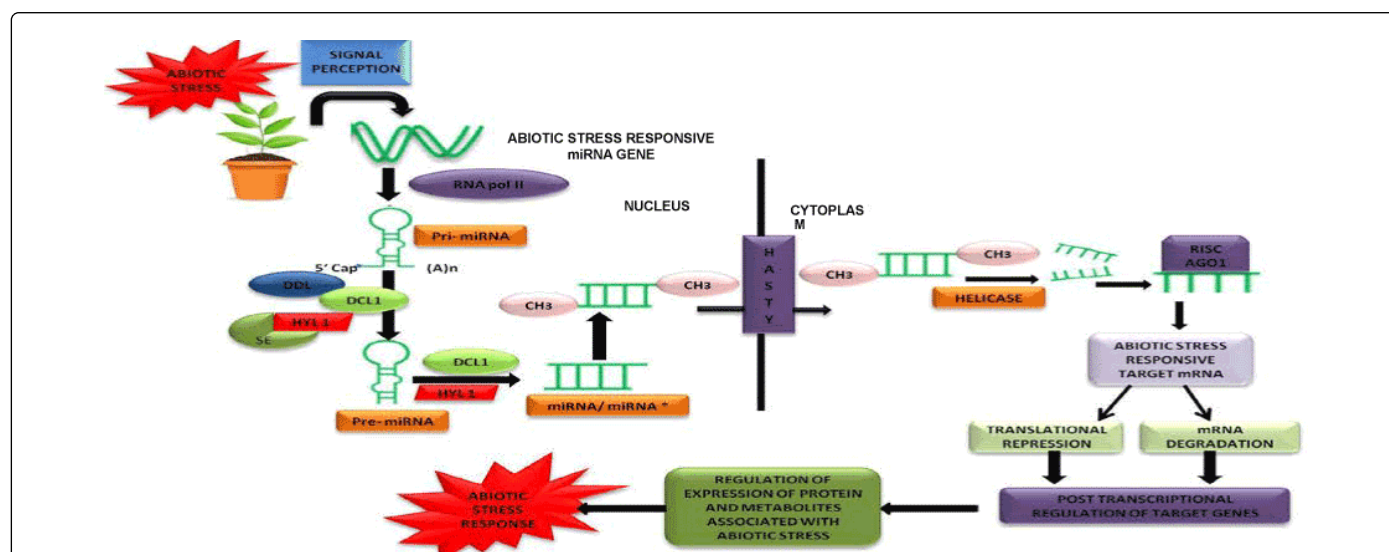


Figure 1: Pathway showing post transcriptional regulation mediated by abiotic stress responsive miRNA genes. Abbreviations: Pri-mRNA: Primary mRNA; DDL: Dawdle; DCL1: Dicer like Protein 1; SE: Serrate; HYL1: Hyponastic Leaves1; RISC: RNA Induced Silencing Complex; AGO1: Argonaute 1

The relationship of expression patterns between a miRNA and its target RNA during the period of stress is dynamic and complex. During stress condition, stress inducible or repressible miRNAs cooperate with transcriptional regulators to control the expression pattern of miRNA-target genes. The expression pattern can be either coherent or incoherent. In coherent regulation, miRNAs along with transcription factors plays a cooperative switch like role. For example, when the plant is exposed to stress, both the miRNA and transcriptional repressor suppress target gene expression. Again in another type of coherent regulation the expression of target gene increases rapidly due to miRNA repression. While in case of incoherent regulation miRNAs functions to fine tune target gene

expression rather than switching it on or off. Thus the functions of stress responsive miRNAs can only be studied by understanding the regulatory interaction within the network [20]. Thus the miRNAs act as a master regulator modulating various gene expressions in plants in response to abiotic stress. Till date the identification of huge number of stress responsive miRNAs might be helpful in developing new strategies to withstand stress thereby improving the stress tolerance in plant. With the future advancement of genomic tools and methods to identify novel miRNAs in various plant species, the number of miRNAs involved in abiotic stress response is increasing thus providing us with a better understanding of miRNAs mediated gene regulation during various abiotic stresses [26,27] [Table 1].

Micro RNA	Abiotic stress					
	Salinity	Drought	Heat	Cold	Oxidative	References
miR156	Ath [*] ,Vun [*] Zma#	Ath [*] ,Ttu [*] , Hvu [*] ,Peu [*] Osa#	Tae [*]	Ptc #	Ath [*]	[5-7,9,12,13,20-22]
miR159	Ath [*]	UK	Tae [*]	UK	Zma [*] , Ath [*]	[6,12,13,23]
miR160	Vun [*]	Peu [*]	Tae [*]	UK	Zma [*]	[5,12,20,22,23]
miR162	Zma [*] ,Vu [*]	Peu [*]	UK	UK	Zma [*]	[5,20,22]
miR165/ miR166	UK	Hvu [*] leaf, Ttu#,Hvu [*] root, Peu#	Ath [*]	Tae [*]	Zma [*]	[5-7,12,20,22]
miR167	Ath [*] , Zma#	Ath [*] , Peu [*]	UK	Osa#	Zma [*]	[1,6,9,12,13,23]
miR168	Ath [*] , Zma# Vun#	Ath [*] , Osa [*] ,Peu [*]	Tae [*]	Ptc [*] , Ath [*]	Osa [*] , Ath [*]	[1,6,9,12,13,23]
miR169	Ath [*] ,Zma [*] , Vun [*] , Osa [*]	Ath [*] , Osa# Mtr# Peu [*]	Tae [*]	Ath [*] , Bdi [*]	Osa [*] , Ath [*] &#	[6,10,12,13,20,21]
miR170/ miR171	Ath [*] , Ptc#	Ath [*] , Hvu [*] leaf, Ttu# ,Osa [*] &# Peu [*] & #	Ptc#	Ptc# Ath [*] Osa#	Zma [*]	[5,6,9,11,22,24]
miR172	UK	Osa [*] ,Peu [*] &#	Tae#	Ath [*] ,Bdi [*]	Ath [*]	[6,9,10,12,13,25]
miR319	Ath [*]	Ath [*] ,Osa [*] &#, Peu [*] &#	UK	Ath [*] , Osa#	UK	[6,9,12,13,25]
miR393	Ath [*] , Pvu [*] , Osa#	Ath [*] , Osa [*] ,Mtr [*] , Pvu [*]	Tae [*]	Ath [*]	UK	[6,10,12,13,20]
miR396	Ath [*] ,Osa [*] , Zma#	Ath [*] , Osa #, Ttu [*] , Peu [*] &#	UK	Ath [*]	Zma [*]	[1,6,9,12,13,23]
miR397	Ath [*]	Ath [*] , Osa#, Peu [*]	UK	Ath [*] , Bdi [*]	Osa [*]	[6,9,10,12,13,25]
miR408	Vun [*]	Ath [*] , Mtr [*] , Hvu [*] , Osa#	UK	Ath [*]	UK	[5,6,9,11,22,24] Hea

Table 1: Expression level of different stress responsive miRNA in plant species

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