Regulation of abiotic stress responses in plant by micro RNA: An Overview

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ABSTRACT

Abiotic stress is one of the primary causes of crop losses worldwide. Plants are sessile organisms, so they have to come up with various adverse environmental conditions. The expression of several genes are up regulated and down regulated in response to stress. Micro RNAs play vital role in regulating the expression of most of the genes during stress, these are small non-coding RNA molecule of approximately 22 nucleotide in length, and play a pivotal role in controlling gene expression during developmental processes and stress responses in plants by attaching themselves to mRNAs for translational repression or target degradation. Plant miRNAs are found to be involved in most of the abiotic stress responses, such as drought, cold, salinity and nutrient deprivation and thus it plays important role in regulating abiotic stress in plants.

Keywords: Plant, microRNAs, abiotic stress

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INTRODUCTION

Micro RNAs are the most abundantly expressed and well-studied class of small RNAs found in plants. It regulates gene expression in a sequence-specific manner. miRNAs are small, noncoding single stranded nucleic acids having 18-25 nucleotides, they down regulate other mRNA molecules at expression level by complementary pairing with it to form RNA duplex or degrading the target mRNA by RISC endonuclease. miRNAs cause epigenetic changes in the organism, which are heritable changes. In plants miRNAs are known to play focal roles in a variety of physiological and developmental processes, such as organ development, flower development, genome maintenance, phase transition, and response to different biotic and abiotic stresses reprogramming. The involvement of miRNAs in the phenomena of plant growth and development has been a topic of research since the last few decades and due to their high throughput studies of their mechanism in biological system has led to the discovery of a large number of miRNAs that have a role to play in regulating stress in plant. It was in 1993, that Victor Ambros, Rosalind Lee and Rhonda Feinbaum identified miRNA in Caenorhabditis elegans during larval development lin-4 gene which codes for a 22 nucleotide miRNA that controls the abundance of Lin 14 protein during larval development and its timing. It is in 2003, ten years later after the discovery in C.elegans that miRNAs were identified in plants (Arabidopsis) confirming their presence in both animals and plants. In 2006, it has been recorded that about 117,178 and 97 types of miRNA molecules have been identified in Arabidopsis, rice, Zea may respectively.

BIOGENESIS OF mi RNAs

There are several genes which have been characterized that code for the microRNAs. Majority of such genes are transcribed regardless of other nearby genes as individual units. Introns of expressing and non-expressing genes also code for miRNAs which account for about 40% of the miRNA genes. The regulation of protein translation by the miRNAs is highlighted by the fact that some of the miRNA genes are transcribed along with the protein coding genes that co-ordinate with each other to control the level of gene expression within an organism to maintain a suitable homeostasis. In the miRNA pathway, RNA polymerase II binds to the promoter of the miRNA genes and transcribes it to produce the primary miRNA (pri-miRNA), which is usually a long sequence of more than several hundred
nucleotides\textsuperscript{5,10}. Pri-miRNA is then processed to a stem loop structure intermediate called miRNA precursor or pre-miRNA by a Dicer homolog called Dicer-like1 (DCL1)\textsuperscript{5,12}. Plant miRNAs are then again cut by DCL1 in the nucleus to form miRNA duplex. The process of formation of mature miRNA duplex from miRNA by DCL1 is also helped by a double-stranded RNA binding protein, HYPONASTIC LEAVES1 (HYL1)\textsuperscript{3,5}. HYL1 is a nuclear protein present in a protein complex\textsuperscript{13}. The mature miRNA duplex on both the strand contains two nucleotide overhangs at the 3’ end.

Addition of methyl groups to the 3’ ends makes miRNA duplex more stable. HUA ENHANCER1 (HEN1), a small RNA-specific methyltransferase is responsible for the methylation of the last nucleotide of 3’ end\textsuperscript{14}. Exonuclease degrades the miRNAs, however miRNA evade this by methylation process which renders the miRNA inefficient to be used as primer for the RDRP (RNA Dependent RNA polymerase) producing dsRNA\textsuperscript{14}. A nuclear exporting protein HASTY (HST), the plant ortholog of Exportin-5 is responsible for the transportation of mature miRNA duplex from nucleus to the cytoplasm\textsuperscript{8}. The mature miRNAs are then finally loaded into RNA-induced silencing complex (RISCs). AGO (ARGONAUTE) proteins are catalytic centers of RISCs. AGO proteins have two domains: a\textasciitilde20 kDa N-terminal PAZ domain and a\textasciitilde40 kDa C-terminal PIWI domain. In the PAZ domain there is a hydrophilic cleft which binds to the 3’ end of single-stranded RNA molecules; PIWI domain has a structure similar to that of RNase H. Based on the different type AGO protein and the type of incorporated miRNAs, the miRNA loaded RISCs bind to the target mRNA in a sequence specific manner and resulting into the cleavage of target\textsuperscript{16} or prevention of the translation process\textsuperscript{17}, or the remodeling of chromatin complex\textsuperscript{18}. Most
of the target mRNAs in plants contain only one complementary site and corresponding miRNAs complementarily bind to this site and cleave the target mRNAs\textsuperscript{5}.

**miRNAs IN PLANT ABIOTIC STRESS**

Plants are being sessile; they have to cope up with various deleterious environmental stress conditions such as drought, extreme temperatures, salinity, heavy metals toxicity, nutrient deficiency, water logging. The negative impact of non-living factor on living organisms in a specific environment is defined as abiotic stress. It is of major importance that plants respond to various stress conditions, to be able to survive and reproduce via the production of seeds. Abiotic stress inflicts various harmful effects at the cellular and molecular levels also, a fast and extensive molecular reprogramming both at the transcriptional and post-transcriptional level is highly essential to recover from the stress effects. To cope with these stress conditions, plants respond by reprogramming gene expression, which results in osmolyte accumulation for osmotic adjustment, up-regulation of antioxidant pathways for reactive oxygen species (ROS) homeostasis, minimizing, as well as repairing the damage caused to the cellular constituents including DNA, proteins and membranes, and maintaining processes that sustains cellular homeostasis under stress\textsuperscript{19}. Transcription factors (TF) are the master regulators which can control many genes at a time and are involved in abiotic stress response\textsuperscript{20,21}.

**miRNAs IN DROUGHT**

Dearth of water is increasing worldwide and resulting into drought stress. The dominant form of agriculture throughout the world is rain-fed agriculture and drought stress is a critical environmental factor which occurs frequently under such condition. By the application of high-throughput technologies such as genome wide gene expression and proteomics several genes are identified that are altered during drought condition\textsuperscript{22,23}. It has been recently found that during drought condition expression of several micromanagers of gene expression (miRNAs) are altered whether by up regulation and down regulation\textsuperscript{24,25}. In plants like Arabidopsis, rice, and *Populus trichocarpa* miRNA-expression profiling has been performed under drought stress. The expression of *miR393*, *miR319*, and *miR397* are up regulated in
response to dehydration in Arabidopsis. In Arabidopsis, miR396, miR168, miR167, miR165, miR319, miR159, miR394, miR156, miR393, miR157, miR158, and miR169 were shown to be drought responsive. Several miRNAs are identified (miR157, miR167, miR168, miR171, miR171, miR408, miR393 and miR396) with the aid of a more comprehensive array-based analysis technique, which were also up-regulated in drought-stressed Arabidopsis. In rice, microarray analysis of miRNA expression profile under drought stress showed significant up-regulation of miR169g, the only member of miR169 family and the induction of miR-169g was more prominent in roots than in shoots. Sequence analysis revealed that miR-169g may be regulated directly by DRE binding transcriptional factors, while miR393 was transiently induced. In drought-challenged rice a genome-wide identification and analysis of miRNAs were carried out during different developmental stages, from tillering to inflorescence formation, using a microarray platform. The consequences of analysis showed that 16 miRNAs (miR156, miR159, miR168, miR170, miR171, miR172, miR319, miR396, miR397, miR408, miR529, miR896, miR1030, miR1035, miR1050, miR1088, and miR1126) were significantly down regulated in response to drought stress and also found that, 14 miRNAs (miR159, miR169, miR171, miR319, miR395, miR474, miR845, miR851, miR854, miR896, miR901, miR903, miR1026, and miR1125) were significantly up-regulated under drought stress. It was found that the expression levels of miR1446a-e, miR1444a, miR1447 and miR1450 were significantly reduced, while miR1711-a, miR482.2, miR530a, miR827, miR1445, and miR1448 were slightly down regulated in response to drought stress. miRNAs expression patterns were investigated in drought-resistant wild emmer wheat (Triticum turgidum) using a plant miRNA microarray platform. In unstressed Triticum 205 miRNAs were used for the detection of miRNA expression levels while 438 miRNAs in drought-stressed were used for detection. Among all the miRNAs, 13 miRNAs (miR1867, miR896, miR398, miR528, miR474, miR1450, miR396, miR1881, miR894, miR156, miR1432, miR166, and miR171) shown to be differentially regulated in drought condition.

miRNAs IN NUTRIENT STRESS

To maintain normal growth and development of plants depends on the
homeostasis of nutrients, any insufficient or excess of nutrients negatively impacts plant growth and development\textsuperscript{31}. To understand the processes of nutrient acquisition, assimilation and metabolism and adaptations in response to low fertility at the molecular regulation level several systematic and integrative genomic approaches have been applied. The association between plant nutrients and regulation mediated by miRNAs were known from the findings of several experiments\textsuperscript{32,33,34}. Sulphur is one of the essential macronutrients; it is transported to the plants in the form of sulfate and has a structural role in protein folding\textsuperscript{35}. miR395 is involved in sulfate homeostasis, during insufficient availability of sulphur miR395 down-regulates low affinity sulfate transporters (AST68) and ATP sulphurilases (APS1, APS3 and APS4) which are involved in sulfate translocation and sulfate metabolism\textsuperscript{32}.

Phosphate (Pi) is one of the important components of plant nutrients and is required for the synthesis of nucleic acids and membrane lipids. Phosphorus is taken up by plant roots in the form of Pi (HPO$_4^{2-}$)\textsuperscript{36,37}. Phosphate homeostasis is maintained by the miR399 regulation. During Phosphate starvation leads to the activation of miR399 by Pi responsive gene (PHR1), which down regulates the phosphate 2 gene (PHO2)\textsuperscript{38}. It was suggested from several studies suggest that miR399 acts as a phosphate starvation signal that is translocated from shoot to root where it promotes phosphate uptake by down-regulating PHO2, which encodes E2-UBC24 (a putative ubiquitin-conjugating enzyme)\textsuperscript{34}. Recent studies in Arabidopsis and rice found that some miRNA species can potentially target and degrade their own precursor to inhibit miRNA accumulation\textsuperscript{38}.

Metals like copper and iron are also incumbent micronutrients to plants. Copper is an essential a component of plastocyanin (essential component in photosynthetic and respiratory electron transport chains) and is also a cofactor for Cu/Zn Superoxide dismutases, laccases and the ethylene receptor\textsuperscript{39}. During copper deficiency conditions the levels of miR398 were up-regulated resulting in to the down-regulation of two Cu/Zn superoxide dismutase mRNA targets (CSD1 and CSD2)\textsuperscript{40}. Recently, found that the miRNAs, miR397, miR408 but not miR857 were conserved among several plants including Arabidopsis,
rice and poplar\textsuperscript{32,41,42}. miR397, miR408 and miR857 were also found to be upregulated under low copper conditions\textsuperscript{39}. In Arabidopsis, several miRNAs were upregulated in response to low iron levels\textsuperscript{43}. When plants are under iron deficiency conditions, a member of the miR854 family is induced, which is conserved in animals also\textsuperscript{43,44}.

**miRNAs IN OXIDATIVE STRESS**

Stress-induced reactive oxygen species (ROS) include superoxide radicals (O\textsuperscript{2−}), hydrogen peroxide (H\textsubscript{2}O\textsubscript{2}) and hydroxyl radicals (OH\textsuperscript{−}) are produced in metabolically active organelles (e.g., chloroplast, mitochondria) in plant cells and excessive accumulation can lead to oxidative stress often observed under diverse biotic and abiotic stresses. ROS accumulation is counteracted by intrinsic antioxidant systems in plants that include a variety of enzymatic scavengers\textsuperscript{45}. The enzymatic scavenging mechanism is comprised of superoxide dismutases (SODs), peroxidases and catalases. Among these SODs are most important enzymes and constitute the first line of defense against highly toxic superoxide radicals. SOD converts highly toxic superoxide radicals (O\textsuperscript{2−}) into less toxic hydrogen peroxide (H\textsubscript{2}O\textsubscript{2}), which in turn is detoxified by peroxidases and catalases\textsuperscript{46}. The induction of superoxide dismutase genes in plants during diverse abiotic stress conditions has been well documented. In Arabidopsis, miR398 family is encoded by three loci (MIR398a, MIR398b, and MIR398c). miR398 targets two closely related Cu/Zn-SOD genes: cytosolic CSD1 and chloroplast-localized CSD2\textsuperscript{32}. miR398 was downregulated under oxidative stress conditions. The lack of CSD1 and CSD2 expression in unstressed plants depends on miR398-mediated posttranscriptional regulation, and the stress induction of CSD1 and CSD2 mRNA is mediated by the downregulation of miR398.

**miRNAs IN COLD STRESS**

Most organisms require an optimum temperature for optimal growth and can only tolerate minor fluctuations. However, fluctuations beyond a certain threshold level leads to high temperature or low temperature stress. Low temperature stress, including chilling and frost, results into the loss of crop yields worldwide by damaging tissue and delaying the growth of plants\textsuperscript{47}. In different species of plant during cold
stress, altered expression of miRNAs, like numerous protein-coding genes has been reported. Under cold stress, the expression of several miRNAs has been examined in Arabidopsis\(^{26,48}\). In all three species, miR397 and miR169 were upregulated and miR172 was upregulated in Arabidopsis and Brach podium. In Arabidopsis, several miRNAs (miR165 / 166, miR393, miR396, and miR408) were induced under cold stress, while other miRNAs (miR156/157, miR159/319, miR164, miR394, and miR398) showed either transient or mild regulation under cold stress\(^{48}\). In Populous, overall fifteen miRNAs (e.g., miR168a,b, miR477a,b) were found to be upregulated while four other miRNAs (miR156g-j, miR475a,b, miR476a) were downregulated under cold stress\(^{29}\). In Brach podium distachyon, by deep sequencing 28 cold response miRNAs were identified\(^{49}\). Rice (Oryza sativa) is one of the most economically important crops in the world and its yield is frequently affected by cold stress. The complete genome sequence of rice is known, so it is used as a model system for cold-sensitive plants. Recently, in rice 414 miRNAs have been identified\(^{50}\), among them 37% are non-conserved in Arabidopsis and the other plant species.

### miRNAs IN SALT STRESS

About 6% of the total arable land is affected by the presence of excess salt in the soil\(^{51}\). Moderate salt stress can reduce crop yields substantially whereas severe salt stress threatens the survival of crop plants. Numerous genes and pathways in plants are differentially regulated under salt stress\(^{23}\). In Arabidopsis, in response to salt stress miR156, miR158, miR159, miR165, miR167, miR168, miR169, miR171, miR319, miR393, miR394, miR396, and miR397 were upregulated, but the accumulation of miR398 was decreased\(^{26}\). In Populus trichocarpa, miR482.2 and miR1450 were upregulated,
however miR530a, miR1445, miR1446a-e, miR1447, and miR171l-n were down regulated during salt stress. Arenas-Huertero et al. observed in Phaseolus vulgaris, that accumulation of miRS1 and miR159.2 were increased in response to NaCl addition\textsuperscript{52}. Later it was found that the two members of the miR169 family, miR169 g and miR169n, were strongly up-regulated in rice\textsuperscript{32}.

**miRNAs IN UV-B RADIATION**

Ultraviolet-B (UV-B, 280–320 nm) radiation on the earth’s surface has been increasing due to day by day depletion of the stratospheric ozone layer\textsuperscript{53}. To identify Arabidopsis miRNAs which are induced by UV-B radiation a computational approach was used and from the study 21 miRNAs belonging to 11 miRNA families were identified\textsuperscript{54}. The miRNAs upregulated under stress were miR156/157, miR159/319, miR160, miR165/166, miR167, miR169, miR170/171, miR172, miR393, miR398, and miR401\textsuperscript{54}. Some of the miRNAs which were upregulated by UV-B radiation in Arabidopsis (miR156, miR160, miR165/166, miR167, miR398, and miR168) were also upregulated by UV-B radiation in Populus tremula\textsuperscript{55}. Remarkably, three families of miRNAs (miR159, miR169, and miR393) which were predicted to be upregulated in Arabidopsis and were down regulated in Populus tremula, suggesting that some responses to UV-B radiation stress may be species specific.

**Conclusion**

Several thousand of stress regulated genes have been identified over the past two decades by the application of high-throughput technologies such as genome wide gene expression and proteomics approaches. With the recent identification of miRNAs as components of stress response, it has been cleared that a miRNA regulation can lead to complex downstream effects on gene expression and regulation. The understanding of post-transcriptional gene regulation by small RNAs such as miRNAs under abiotic stress is crucial for understanding and improving stress tolerance in crop plants.

From literature survey it was found that miR393 is one of the key miRNA expressed during stress responses. It was observed that altered expression of miR393 was found in A. thaliana, Oryza sativa, Phaseolus vulgaris and other plants under different stress conditions such as drought, salinity, low temperature
etc. So miR393 can be used as a promising candidate for manipulating the expression pattern of different stress inducible genes and hence tolerance can be achieved against broad abiotic stress conditions by transgenic approach.

References


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