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Salt stress tolerance in plants: the role of miRNAs

Abstract

Soil salinity is a challenge for agriculture worldwide as crop plants are more sensitive to it compared to non-crops. Development of salinity tolerant crop plants through breeding approaches has been quite a task to the breeder so far because of quantitative nature of the trait. Hence, great emphasis has been given to understand the mechanism underlying salt tolerance in plant so that crop cultivars tolerant to salinity may be developed by biotechnological interventions. The studies on this have basically been directed into two paths; firstly, identification and functional characterization of the genes encoding the effector proteins, including those involved in maintenance of ion homeostasis, osmolytes accumulation and reactive oxygen scavenging and secondly, understanding the regulatory aspects of the effector genes. Accordingly this review describes the issues related to salinity in agriculture with emphasis on understanding on the current developments in regulatory aspects of salt tolerance. In past decade much emphasis has been given on understanding salt tolerance in terms of its regulation by miRNAs, the robust master regulator of the level of information to be carried out from gene to protein. The level of information that is currently available on salt-responsive miRNAs and their targets has enabled the researchers to look into their functional significance in salt tolerance by developing plants over expressing the concerned miRNAs. However, currently our understanding on the involvement on salt tolerance, or a biotic stress tolerance in general is far from complete, and hence requires further investigation, focusing particularly on salt responsiveness of miRNAs in halophytes and study of their expression pattern and targets across species.

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Introduction

Salinity as an a biotic stress of global importance

Environmental disturbances adversely affect the normal physiological functioning of an organism.[1] It can be the fluctuation of temperature, increase in salinity level, water deficit or sub mergance conditions, light-related variations, decre ase in soil moisture content, etc. Being sessile, plants cope with the adverse environment by evolving stress tolerance mechanisms. Stress tolerance mainly involves biochemical, molecular and genetic mechanisms which are ultimately controlled by genes. The regulation of gene expression temporally and spatially in response to environmental cues is an important factor in plant survival and adaptability leading to development of an ecotype.[2] A biotic stresses are the main cause of huge crop yield loss worldwide, and among these high salinity stress is a major threat to agriculture.

Salt stress tolerance and miRNAs

NaCl is the most pervasive and highly abundant salt on the surface of earth. Increased salt levels leads to osmotic stress which subsequently leads to ion toxicity in the plants. This severely deteriorates the plant's ability to take up nutrients and hence leads to nutrient stress and hampered shoot growth and organ development. The plants have evolved various interlinked mechanisms leading to osmotic tolerance and ion exclusion. Various genes and transcription factors get altered to great extents in order to complement the ill-effects of the stress.

Out of these important tolerance mechanisms, small RNAs establish themselves as huge players in post transcriptional regulation of gene expression. These sRNA are either positively regulated by stress, where they enhance the suppression of the genes serving as negative regulators of stress tolerance. Or negatively regulated where the target is positive regulator of stress causing more accumulation of gene product.[3] Amongst these regulatory small RNAs, MicroRNAs (miRNAs) have generated considerable excitement recently.[4]

Tiny yet potent regulators, the miRNAs

The expression of a gene gets regulated at various levels by a large number of molecules. One such class of regulators involve tiny ~21 to 24nt long small-RNA molecules called the miRNAs, which regulate the expression of genes at the post-transcriptional and translational level. The biogenesis of miRNAs gets initiated with the *MIR* genes[5] transcribing to primary-miRNAs (Figure 1). These pre-miRNAs fold up to characteristic stem-looped forms where one of the arms bears the mature miRNA and the opposite arm contains the miRNA* sequence, which get cleaved as a duplex.[6] The ends of the duplex get ethylated following transportation of the duplex from the nucleus to the cytoplasm with the aid of HASTY protein.[7] In the cytoplasm the two strands separate with the loading of mature miRNA strand on to the RISC,[8] which is a multi-subunit assembly of AGO protein[9,10] and the mature miRNA strand which possesses slicer/rib nuclease activity.

The miRNAs target their cognate mRNAs with perfect or near-perfect complementarily leading to cleavage, translational repression or sometimes de-adenylation. Therefore, the miRNAs negatively regulate the levels of target mRNA and consequently the proteins which are coded by them. They do not however, regulate the *MIR* genes from where they originate due to the sequence similarity rather the complementarily. There are a number of conserved miRNA families which are found to be involved in various stress responses and major developmental processes. However, the abundance of newer miRNA sequences is sticking due to their universal involvement, particularly in individual stress conditions or specific developmental stage. Till date 8675 mature miRNAs have been submitted in the miRbase database (version: 21) with *Medicagotrunculata* leading the chart with 756 reported mature miRNAs followed by 713 mature miRNAs in *Oryza sativa*. Recent studies on various plants have revealed important role of miRNAs in salt stress responses. Sunkar & Zhu[11] for the first time created small RNA libraries and sequenced them from plants treated with various stress conditions such as salinity, cold, dehydration, and abscise acid (ABA). Cloning of small RNAs from stressed plants resulted in the identification of 15 novel miRNAs in addition to some siRNAs. The result showed that drought, cold and salinity stress strongly induces miR402 expression while other miRNAs such as miR319 is induced by either cold or other stresses.[11] The various other important miRNAs involved in salinity stress, in different plant species are enlisted in Table 1.

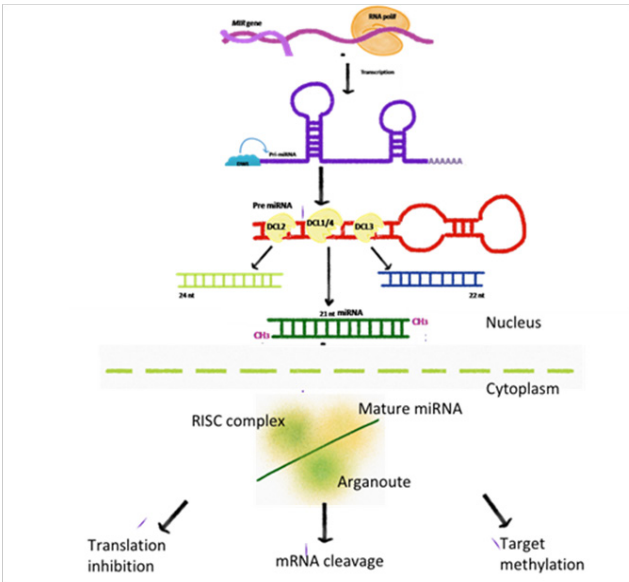

Figure 1 Biogenesis of miRNA and its action.

Many studies have shown that one miRNA may respond differently to salt stress depending on the plant species. For example, salinity stress induces the expression of miR156 in *Arabidopsis*[12] but decreases in maize.[13] Similarly salinity treatment up-regulated the expression of miR396 in *Arabidopsis*[12] and maize[13] but down regulated in rice.[14] The expression of miRNAs has also been studied in salt stress sensitive and salt tolerant plant varieties to investigate the importance of miRNAs in plant salt stress responses. Comparative analysis between salt sensitive maize genotypes Huangzao4 and salt tolerant maize genotype NC286 identified 98 miRNAs from 27 families, among these miR156, miR164, miR166, miR167 and miR396 family members were down-regulated, whereas miR162, miR168, miR395 and miR474 families were up-regulated in salt stressed maize roots.[13] Microarray experiment on two cotton cultivars (salt tolerance SN-011 and salt sensitive LM-6) demonstrated that miR156, miR169, miR535 and miR827 were significantly up-regulated in LM-6, while miR167, miR397 and miR399 were down regulated in it.[15] Differential expression of *Ath*-miR169b, *Osa*-miR1432, *Hv*-miRx5, *Hv*-miR166b/c was seen in well-watered and drought-induced varieties in Barley along with their putative targets.[16] All these studies suggest that comprehensive analysis of miRNA response to salt stress in closely related genotypes with contrasting stress sensitivities would provide better insights into miRNA-guided gene regulation and such strategies can be used for improving the salt stress tolerance of crop plants.

Table 1 List of reported salt stress responsive miRNAs and their targets in plants

Plant species	miRNA	miRNA Abundance	Target	References
Arabidopsis thaliana	miR156	Up-regulated	Squamosa promoter-binding protein-like11	12
	miR158	Up-regulated	Pentatricopeptide repeat-containing protein(PPR)	
	miR159	Up-regulated	MYB and TCP transcription factors	
	miR165	Up-regulated	Class III HD-ZIP transcription factors	
	miR167	Up-regulated	Auxin response factor	
	miR168	Up-regulated	ARGONAUTE 1 (AGO1)	
	miR169	Up-regulated	CCAAT-binding transcription factor (CBF-B/NF-YA) family protein	
	miR171	Up-regulated	Scarecrow transcription factor	
	miR319	Up-regulated	TCP transcription factors	
	miR393	Up-regulated	F-box protein; bHLH (basic helix–loop–helix) transcription factor	
	miR394	Up-regulated	F-box family protein	
	miR396	Up-regulated	Growth regulating factor 2 transcription factor Rhodenase-like protein	
	miR397	Up-regulated	Laccases 2	
	miR398	Down regulated	InterPro domain Protein of unknown function DUF266,	
Zea mays	miR156	Down regulated	SBP-domain protein	13
	miR162	Up-regulated	Dicer-like (DCL)	
	miR164	Down regulated	NAC domain protein NAC1	
	miR166	Down regulated	Homeo domain leucine Zipper protein (HD-ZIP)	
	miR167	Down regulated	Auxin response factor	
	miR168	Up-regulated	ARGONAUTE 1 (AGO1)	
	miR395	Up-regulated	ATP sulfurylase	
	miR396	Down regulated	Cytochrome oxidase subunit 1	

Table Continued...

Plant species	miRNA	miRNA Abundance	Target	References
	miR171	Down regulated	Scarecrow-like protein	
	miR482.2	Up-regulated	Disease resistance protein	
	miR530a	Down regulated	Zinc knuckle (CCHC-type) family protein,	
Populus trichocarpa	miR1445	Down regulated	Dihydropyrimidinase	26
	miR1446a-e	Down regulated	GCN5-related N-acetyltransferase (GNAT) Family protein,	
	miR1447	Up-regulated	Ankyrin repeat family protein,	
	miR1450	Up-regulated	Leucine-rich repeat transmembrane protein kinase	
	miR319	Up-regulated	TCP transcription factor	
	miR393	Up-regulated	APETELA 2 (AP2)	
	miR394	Up-regulated	F-Box protein	
	miR395	Up-regulated	ATP sulfurylase	
Populus tomentosa	miR396	Down regulated	Growth regulating factor	27
	miR398	Up-regulated	Copper superoxide dismutase	
	miR399	Up-regulated	E2-ubiquitin conjugating enzyme	
	miR408	Up-regulated	Plasma cell leukemia	
	miR1450	Down regulated	Ankyrin repeat L-RTMK	
	miR156c	Down regulated	Squamosa promoter-binding protein	
Solanum linnaeanum	miR167a	Down-regulated	Annexin 1	28
	miR397a	Up-regulated	Laccase	
	miR5300	Down-regulated	Nucleotide-binding site-leucine-rich repeat	
	miR166g-3p	Down-regulated	S-adenosylmethionine-dependent methyltransferase	
Raphanus sativus	miR169b	Down-regulated	Nuclear transcription factor Y subunit A-3	29
	miR172c	Down-regulated	AP2-like ethylene responsive transcription factor SNZ	

In rice, three members of miR169 family-miR169g, miR169n and miR169o as well as miR393 were found to be upregulated during salinity stress[17,18] which specifically cleaves the gene transcript of NF-YA transcription factor which is evolutionary conserved in a wide range of organisms from yeast to humans. In *Arabidopsis*, miR169 was also induced by salinity stress.[19] A contrasting feature was observed between miR398 and its target genes CSD1 and CSD2 during salinity stress. Microarray analysis in *Populus tremula* demonstrated dynamic regulation of miR398 abundance during salt stress with an initial increase followed by decrease and then again increase.[20] Such active regulation of miR398 was absent in *Arabidopsis*, in which the abundance of miR398 was decreased and its target genes (CSD1 and CSD1) abundance was increased during salt stress.[21] The expression level of miR168 was found to be upregulated under salt stress in both *Arabidopsis*[12] and maize.[13] The miR168 regulates the expression of AGO1 through an auto-regulatory mechanism to maintain homeostasis of AGO1.[10] Since, AGO1 is a key component of RISC and required for miRNA function, any variation in miR168 expression has potential influence on the function of other miRNAs.

In contrast to glycophytes, halophytes can grow well in high saline conditions and are well suited candidates to study salt adaptation mechanisms in plants. Hence, investigating salt responsive miRNAs from halophytes may help us in better understanding of molecular mechanisms of salt adaptation in plants. Although salt responsive miRNAs have been profiled in various glycophytes, they have been analyzed in only a few halophytes. Dassanayake et al.,[22] 2010 for the first time computationally predicted 12 conserved miRNA families using mangrove transcriptome database and proposed regulatory models for these miRNAs by comparing distribution and positions of miRNA targets in mangrove plants and *Arabidopsis*. In *Avicennia marina* (Red sea mangrove), total 193 conserved miRNAs and 23 novel miRNAs were identified by using small RNA deep sequencing.[14] Further, experimental validation of predicted miRNA target genes of both novel (miR2.1, miR2.2, miR3.1, miR3.5, miR4, miR7, miR8, miR11 and miR12) and conserved (miR156, miR159, miR160, miR166, miR170, miR390, miR397 and miR398) miRNAs using 5'-RACE PCR followed by sequencing revealed target cleavage occurs at an expected position (Position 10 to 11). Their results suggested that the expression profiles of salt-responsive miRNAs and their target genes in *A. marina* may help to elucidate the important role of miRNAs in mangroves, including their response to a biotic stress.[23]A recent study on *Halostachys caspica*, identified a total of 170 conserved miRNAs; among these miRNAs, 48 were significantly down regulated and 31 were significantly up-regulated by salinity stress.[24] They also identified 102 novel miRNAs; among them, 13 miRNAs were significantly down regulated and 12 miRNAs were significantly up-regulated by salinity. Further investigation on miRNA-target interaction by GO and KEGG analysis suggested many miRNAs involved in stress-related pathway. The expression profiles of conserved and novel miRNAs during salinity stress and between the shoots and roots of *Salicornia europaea*, a salt marsh euhalophyte, revealed that they may play an important role in salt tolerance by regulating their downstream targets.[25] Hence, all these studies of salt-responsive miRNAs in halophyte plants may elucidate salt tolerance mechanisms and could be used to improve salt tolerance in crops and other plants.

MicroRNAs, a new target for improving plant tolerance to salt stress

Understanding miRNA-guided stress regulation followed by the use of this knowledge to engineer stress tolerant plants is unavoidable. Currently, many studies have shown that a biotic stresses induce aberrant expression of miRNAs, thus miRNAs could be used as a target for plant improvement, including enhanced tolerance to multiple stresses.[30] Over expression of miRNA can be achieved by using an inducible promoter or constitutive promoter such as 35S or polyubiquitin promoters that is activated only under particular stress condition. To date, it has been shown in few reports that several miRNAs have been over expressed in multiple plant species and depending on their target genes they exhibited either higher tolerance or sensitivity to salt stress. Most of the transgenic studies provide strong evidence for the use of miRNA-based technology for enhancing plant tolerance to various a biotic stresses particularly salt stress.

Conclusion

Research on understanding the mechanism of salt tolerance in plants is being carried out since decades. There is clear understanding of the probable underlying mechanisms, such as maintenance of cellular ion-homeostasis with regard to K+ and Na+, accumulation of osmolytes like proline and glycinebetaine for the maintenance of cellular osmotic potential, and effective removal of reactive oxygen species from cells and tissues. However, these processes require coherent participation of a number of genes, the expression of which is controlled by interplay of complex mechanisms involving signal transductions, cis- and trans- elements interaction, chromatin modifications, protein modification and localization, etc. So far it has not been possible to establish the linkage between various biochemical processes that enable the plant to achieve salt tolerance at the physiological level. In fact this is the major factor that has frustrated the researchers worldwide in their attempt to make a plant salt-sensitive plant salt tolerant. MiRNAs, the master regulator of gene expression is ray of hope in this regard, and that is why huge data on regulatory role of miRNAs in a biotic stress tolerance could be generated in a short stretch of time since their discovery. Although it is a long way for finally understanding the regulatory processes governing the salt tolerance, but identification of the salt-responsive miRNAs and their targets, and establishing the function of these targets is certainly going to yield promising results, which could be valuable contributions to the ongoing attempts of introducing the salt tolerance trait in the plant species of interest.

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Conflicts of interest

Author declares that there is no conflicts of interest.

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