Chapter 2
MicroRNAs and Their Role in Salt Stress Response in Plants

Satendra K. Mangrauthia, Surekha Agarwal, B. Sailaja, M. Sheshu Madhav, and S.R. Voleti

Abstract MicroRNAs (miRNAs) are a subset of endogenous approximate 22 nucleotide (nt) small non-coding regulatory RNA molecules that regulate gene expression post-transcriptionally, by mediating mRNA degradation or translational repression in a sequence specific manner. These small regulatory molecules are involved in regulating the intrinsic normal growth of cells and development of organisms as well as in maintaining the integrity of genomes. The plant miRNA research gained momentum, 2002 onwards, which was accompanied by the discovery of plant proteins involved in miRNA biogenesis. Early discovery of miRNAs has been implicated in the regulation of developmental processes. Since then much has been discovered about their involvement in plant responses to adverse environmental conditions, including abiotic stress. Various approaches of miRNAs discovery such as cloning, deep sequencing and prediction using bioinformatic tools have been adapted to learn more about the miRNA expression patterns during stress. The master regulators such as miRNAs having important role in salt stress response are very much crucial to understand the molecular regulation of stress adaptation. Many target genes of miRNAs encode transcription factors, each of which further regulates a set of downstream genes and affect physiological responses. This chapter contains a concise account on historical importance of miRNAs discovery. The miRNA biogenesis pathway and the associated proteins are also discussed along with the tools of miRNAs prediction and identification. In addition, the role of plant miRNAs and their target in plant metabolism and in particular salt stress is elaborated. With the growing knowledge on salt responsive miRNAs, the efforts to develop salt stress tolerance using miRNAs are also given.

Keywords miRNA • Biogenesis • Target genes • Deep sequencing • Regulation

S.K. Mangrauthia (✉) • S. Agarwal • B. Sailaja • M.S. Madhav • S.R. Voleti
Directorate of Rice Research, Hyderabad 500 030, Andhra Pradesh, India
e-mail: skmdrr@gmail.com

P. Ahmad et al. (eds.), *Salt Stress in Plants: Signalling, Omics and Adaptations*,
DOI 10.1007/978-1-4614-6108-1_2,

Plant development, metabolism and stress responses greatly rely on the correct and timely regulation of gene expression that ultimately affect the plant metabolites such as osmolytes and antioxidants (Koyro et al. 2012). A large class of plant genes is regulated by stresses such as diseases, insects, drought, cold, heat and soil salinity. Salinity is defined as the concentration of dissolved mineral salts present in the soils and waters. The dissolved mineral salts consist of the electrolytes of cations and anions. The major cations in saline soil solutions consist of Na^+, Ca^{2+}, Mg^{2+} and K^+ and the major anions are Cl^-, SO_4^{2-}, HCO_3^-, CO_3^{2-} and NO_3^-. Other electrolytes contributing to soil and water salinity include B, Sr^{2+}, SiO_2, Mo, Ba^{2+} and Al^{3+} (Hu et al. 2007). Salinity is generally a problem in arid and semi-arid areas where evapo-transpiration exceeds annual precipitation, and in such areas supply of irrigation water is therefore essential to meet water needs of crop plants. The plants get affected due to salinity by two major ways: the capacity of roots to extract water is disturbed due to high concentrations of salts in the soil, and high concentrations of salts within the plant cells can be toxic. These effects can ultimately result in an inhibition of many biochemical, molecular, and physiological processes, for instance, nutrient uptake, assimilation, cell signaling pathways including those that lead to synthesis of osmotically active metabolites, specific proteins, and certain free radical scavenging enzymes that control ion and water flux and support scavenging of oxygen radicals or chaperones. Salinity stress often activates reactive oxygen species (ROS) detoxification forms an important defense against salt stress (Monteiro et al. 2011). Furthermore, salt stress may cause water stress, ion toxicity, nutritional disorders, oxidative stress, alteration of metabolic processes, membrane disorganization, reduction of cell division and expansion, genotoxicity, etc. (Zhu 2007).

The salt stress response is a quantitative trait, involving activation of a large number of specific genes concomitant to the repression in activity of a large number of housekeeping genes (Sahi et al. 2006). Major changes in transcriptional and post-transcriptional events have been noticed to supplement response of plants to salt stress. In order to understand the transcriptional profiling of genes whose expression levels alter in response to salt stress, different EST/cDNA collections have been utilized in plants (Shiozaki et al. 2005). The different studies on genomic and proteomic suggest that overall tolerance to high salt concentration is due to effectors that directly modulate stress etiology or attenuate stress effects and due to key regulatory molecules which are involved in stress perception, signal transduction, and modulation of the effectors' functions (Hasegawa et al. 2000; Sahi et al. 2003). Furthermore, comparative genomics approaches have been employed to understand the progress on the transcript changes in response to high salinity. Multiple mechanisms involved in salt stress perception and tolerance, with perhaps the most important switch being exercised at the transcription level, the importance of post-transcriptional gene regulation is accelerated with the discovery of small RNAs such as siRNA (small interfering RNA) and miRNA (micro RNA).

Post-transcriptional regulation of genes through small RNAs is termed as RNA silencing or RNA interference (RNAi). It is a versatile, complex gene regulation and defense mechanism known to be operative in most, if not all eukaryotic organisms (Tomari and Zamore 2005). RNA silencing operates through a set of core reactions

that are triggered by double stranded RNA (dsRNA), which is processed into 21–24 nt small RNA duplexes by the RNase III enzyme Dicer and its homologues (Bernstein et al. 2001). These small RNAs, in turn, mediate multiple regulatory and defense functions in cells (Brodersen and Voinnet 2006). MicroRNAs and small interfering RNAs are two major types of these small RNAs. Although miRNAs and siRNAs are similar in size (20–24 nucleotides), their biogenesis and modes of action are markedly different. In plants, post transcriptional gene regulation involves miRNAs, generated by Dicer-like 1 (DCL1) from miRNA precursors that are transcribed from miRNA genes (Agarwal et al. 2011).

miRNAs are known to down-regulate plant gene expression at the post-transcriptional level mainly by annealing to reverse complementary sequences, resulting in breakage or translational suppression of the target mRNAs. In addition plant miRNAs direct the methylation of target chromosomal loci to regulate the gene expression at transcription level. Genetic and biochemical studies further enhancing our understanding of the biogenesis and function of miRNAs in plant gene expression and regulation. The plant miRNAs have been identified and characterized to demonstrate that miRNAs play essential roles in growth and development in different plants species (Mallory and Vaucheret 2006). Role of miRNAs in stress responses could be linked from the discovery that biotic and abiotic stress can modulate miRNA levels, together with the alteration in expression level of stress-associated genes as miRNA targets. Now with various genomic tools, it is established that several plant miRNAs play vital roles in plant resistance to stresses. In this chapter we have provided different aspects of miRNAs and their role in salt stress response. The historical importance, enzymatic machinery and biogenesis of plant miRNAs have been covered along with the various tools of miRNA identification.

Historical Perspective of miRNAs

On the basis of the structural similarity between miRNAs and siRNAs, 21–23 nt miRNAs originally described in *Caenorhabditis elegans* that had less complementarity to their target genes (Lee et al. 1993; Wightman et al. 1993), it was then shown that miRNAs could also cause RNA silencing (Olsen and Ambros 1999; Reinhart et al. 2000) and that siRNAs were co-opting this endogenous machinery for RNA silencing. MicroRNAs were discovered by Ambros and colleagues in 1993 during the study of the role of *lin-14* gene in *C. elegans* developmental biology. They observed that LIN-14 protein abundance was regulated by a short RNA product encoded by another gene lin-4. Therefore, lin-4 was the first member of the miRNA family of plants and animals which was identified in *C. elegans* through a genetic screening for defects in the temporal control of post-embryonic development. Most of the genes identified from mutagenesis screens were protein-coding, but lin-4 encoded a 22-nucleotide non-coding RNA that was partially complementary to seven conserved sites located in the 3′-untranslated region (UTR) of the lin-14 gene. At that time it was thought to be a rarity of gene regulation mechanism. Further, these genetic and biochemical interactions

inspired a series of studies showing that the direct, but imperfect, base pairing between lin-4 and the lin-14 3′UTR was essential for the ability of lin-4 to control LIN-14 expression through the regulation of protein synthesis. Next in the year 2000, a second miRNA called *let-7* was characterized using forward genetics in *C. elegans*, which repressed *lin-41* and *lin-57* expression during developmental stage transitions in this worm. *let-7* also encoded a temporally regulated 21-nucleotide short RNA that controls the developmental transition from the larval stage into the adult stage. Both the miRNAs (lin4 and let7) were key regulatory molecules in the pathway of the temporal regulation of larval development in the nematode. The term short-temporal RNA (stRNA) was used when this class of small RNA in *C. elegans* was described in 1993 (Lee et al. 1993; Wightman et al. 1993), but the term microRNA (miRNA) was only coined in 2001. The identification of these miRNAs in *C. elegans* not only provided another vivid example of developmental regulation by small RNAs, but also raised the possibility that such tiny RNAs might be present in other animals and plants species.

The discovery of let-7 miRNA in *C. elegans* in 2000 paved the way for identification of number of miRNAs in both plants and animals. The first miRNA of plant origin was identified in 2002 by cloning approach in *Arabidopsis thaliana* (Llave et al. 2002; Park et al. 2002; Reinhart et al. 2002). Since then, many miRNAs and their target genes have been identified and characterized in various other plants species including rice, Arabidopsis and maize etc. Most miRNA sequences are conserved among different plant species. With the availability of the complete genome sequence of rice, 138 miRNAs representing 20 families were predicted based on sequence conservation with Arabidopsis by using computational methods (Jones-Rhoades and Bartel 2004; Park et al. 2002; Reinhart et al. 2002). Later, many novel miRNAs have been revealed in other plants by direct cloning, traditional sequencing and deep sequencing tools.

It is believed that the miRNA mediated gene regulation is an ancient mechanism of controlling the gene expression and it occurred prior to the emergence of multicellularity. This also suggests that miRNAs must have a common ancestor in evolution (Zhang et al. 2005). Interestingly, two Arabidopsis miRNAs were found conserved in all lineages of land plants, including bryophytes, lycopods, ferns and seed plants. These miRNAs are known to be capable of regulating genes in HD-Zip gene family (Floyd and Bowman 2004). As for as role of miRNAs in plant biology is concerned, initially it was shown that plant miRNAs are involved mainly in developmental regulation such as leaf development, auxin signaling, phase transition and flowering etc. Later it was reported that these miRNAs also play a critical role in biotic as well as abiotic stress response. As it is known that different abiotic stresses may lead to alteration of expression of similar set of genes in plants similarly, different kinds of stresses have also been found to activate responses in similar sets of miRNAs. The first abiotic stress related miRNAs in plants was reported by Sunkar and Zhu (2004). A library of small RNAs was constructed from Arabidopsis seedlings exposed to different abiotic stresses including cold, dehydration, high salt, and abscisic acid (ABA) to identify several new miRNAs that are responsive to abiotic stress. After that, several abiotic stress related miRNAs were identified in different plant species such as *Oryza sativa*, *Brassica napus*, *Glycine max, Medicago truncatula*, *Physcomitrella patens*, *Populus trichocarpa*, *Saccharum officinarum*, *Sorghum bicolor*, and *Zea mays* etc.

Enzymatic Machinery of miRNAs Pathway

Dicer

Dicer is an enzyme that cleaves long double stranded RNA (dsRNA) into short dsRNA and it is the key step in the process of RNAi mediated gene regulation. Dicer represents the class of RNAse III endonucleases and posses five major domains for its dicing activity: (1) RBD (RNA Binding Domain) domain recognizes dsRNA structure; (2) two RNase III domains involved in cleavage of dsRNA into short RNA of ~21–24 nt; (3) PAZ (Piwi, Argonaute, Zwile) domain which binds to 3′-2 nt overhangs of cleaved RNA substrate; (4) C terminal helicase domain required for the dsRNA processing. Different forms of dicer enzymes are involved in the generation of different class of small RNAs. Plants have multiple dicers for instance Arabidopsis has four Dicer like proteins viz., DCL 1, 2, 3 and 4. DCL1 is responsible for miRNA generation while DCL2, DCL3 and DCL4 are required for the siRNA generation. Based on the observation that pri-miRNA levels increase and pre-miRNA levels decrease in the weak *dcl1-9* insertion mutant, DCL1 is believed to be the major player to cleave pri-miRNA to pre-miRNA (Song et al. 2007). Indeed, a dicer enzyme from *A. thaliana,* AtDCL1 is required at several steps in the maturation of the Arabidopsis miR163, and similarly, OsDCL1 appears to play an essential role in miRNA biogenesis in *Oryza sativa* (Kurihara and Watanabe 2004; Liu et al. 2005). Further, six DCLs have been reported in rice which are involved in recruiting different classes of small RNAs in plant gene expression and regulation (Liu et al. 2007).

Dawdle (DDL)

DAWDLE (DDL), an forkhead-associated domain (FHA) containing protein of Arabidopsis is involved in the production of miRNAs and endogenous siRNAs. DDL is an RNA binding protein and is able to interact with (DCL1) and participates in miRNA biogenesis by facilitating DCL1 to access or recognize pri-miRNAs. Unlike mutants of genes known to participate in the processing of miRNA precursors, *ddl* mutants show reduced levels of pri-miRNAs as well as mature miRNAs but the promoter activity of MIR genes is not affected by *ddl* mutations. SNIP1, the human homolog of DDL, is involved in miRNA biogenesis and interacts with Drosha (Yu et al. 2008).

Hyponastic Leaves 1 (HYL1) and Serrate (SE)

Several studies suggest that the dsRNA-binding protein (dsRBP) HYL1 and a C2H2 zinc-finger protein SE are DCL1 cofactors. HYL1 is involved in miRNA but not in siRNA biogenesis. This dsRNA binding protein is a part of a macromolecular complex

involved in miRNA maturation (Vazquez et al. 2004). HYL1 has a function in assisting the efficient and precise cleavage of pri-miRNA through interaction with DCL1. Genetic studies with Arabidospsis indicate that three proteins, the RNase III DCL1, HYL1, and SE, are required for the accurate processing of microRNA (miRNA) precursors in the plant cell nucleus (Dong et al. 2008). It has been shown that in both *hyl1* and *se* mutants the mature miRNA levels are low (Vazquez et al. 2004), and pri-miRNAs accumulate (Song et al. 2007). DCL1 and HYL1 recombinant proteins form a complex in vitro and HYL1 has been reported to interact with SE (Dong et al. 2008).

Hua Enhancer1 (HEN1)

HEN1 has a putative dsRNA-binding motif and a C-terminal methyltransferase domain. It adds a 2′-O-methyl group on the 3′-terminal nucleotide of plant miRNAs and siRNAs to protect them against degradation (Huang et al. 2009b). It has been demonstrated that the *hen1-1, hen1-2,* and *hen1-4* mutations that compromise miRNA metabolism are all in the methyltransferase region. Purified HEN1 protein is able to methylate the miRNA/miRNA* duplex in vitro. HEN1 is highly selective of its substrate and miRNA/miRNA* of different primary sequences can serve as substrates, suggesting that HEN1 recognizes the structure (rather than the sequence) of the duplex produced by Dicer processing of pre-miRNA (Yang et al. 2006).

Argonaute

Argonaute protein (Ago) is the most important component of RISC (RNAi induced silencing complex) which is involved in cleavage or suppression of target mRNA. The small RNAs act like a guide of RISC to reach target mRNA. Argonaute protein possesses two domains viz., PAZ and PIWI domains. In plants and animals, different Ago proteins are members of si and mi RISC. Ago-1 is a part of si-RISC whereas Ago-2 for mi-RISC. Many other Ago proteins have been reported in Arabidopsis until now, Ago-4 generates repeat associated siRNAs (rasi-RNAs) which participate in RNA-directed DNA methylation, Ago-6 has role in DNA methylation and transcriptional gene silencing while Ago-7 has been shown to generate trans acting siRNA (tasi-RNA) and long siRNAs (Naqvi et al. 2009).

Biogenesis of Plant miRNAs

Plant miRNAs, primarily found in genomic regions not associated with protein coding genes (Reinhart et al. 2002), are produced from their own transcriptional units. miRNAs encoded by endogenous MIR genes are ~20–24 nt double stranded small

RNAs (dsRNA) with 5′-phosphate and 3′-hydroxyl groups with 2-nt overhangs. MicroRNA biogenesis requires multiple steps in order to form mature miRNAs from miRNA genes (Kurihara and Watanabe 2004). In a first step, a miRNA gene is transcribed to a primary miRNA (pri-miRNA), which is usually a long sequence of more than several hundred nucleotides. The RNA polymerase II is responsible for transcribing most plant miRNAs (Xie et al. 2005). There are several observations to strengthen the involvement of RNA polymerase II in processing of miRNAs; such as plant pri-miRNAs can be more than 1 kb in length, they are usually preceded by typical TATA box motifs, and that they can undergo canonical splicing, polyadenylation, and capping. The pri-miRNA is processed within the nucleus by a multiprotein complex called the Microprocessor. The second step involves cleavage of the pri-miRNA to a stem loop intermediate called miRNA precursor or pre-miRNA. This step is controlled by the Dicer-like 1 enzyme (DCL1) in plants (Kurihara and Watanabe 2004). The fork head-associated domain protein DAWDLE (DDL) is required for pri-RNA accumulation and also it has been proposed to play an important role in miRNA biogenesis by recruiting predominantly DICER-like protein 1 (DCL1) to pri-miRNA for downstream processing (Yu et al. 2008). The DCL1 together with HYL1 (HYPONASTIC LEAVES 1) and the zinc-finger protein SE (SERRATE) were required for processing of pre-miRNA into miRNA duplex. Most of the Arabidopsis miRNAs are matured in subnuclear bodies by DCL1, while a few appear to be DCL4 dependent (Rajagopalan et al. 2006).

The 2-nt 3′overhang, characteristic of RNase III–mediated cleavage gets methylated by HEN1 (HUA ENHANCER 1), that is recognized by Exportin (in animals) homolog, HST (HASTY), however, there are some reservations about whether miRNA duplexes or mature miRNAs are exported in plants by HASTY to the cytoplasm (Park et al. 2005). This whole process yields a precursor miRNA (pre-miRNA) and ultimately a mature miRNA/miRNA* duplex. In the cytoplasm, miRNAs are unwound into single strand mature miRNAs by helicase. The miRNA strand with relatively lower stability of base-pairing at its 5′ end act as guide molecule to reach the target mRNA and is incorporated into a ribonucleoprotein complex RISC, whereas the other miRNA strand is typically degraded (Du and Zamore 2005). Once incorporated into RISC, the miRNA directs AGO1 (or AGO10) containing RISCs to its target mRNA for cleavage or translational repression on the basis of sequence complementarity. In cases of perfect or near-perfect complementarity to the miRNA, target mRNA scan be cleaved (sliced) and degraded; otherwise, their translation is repressed (Martinez and Tuschl 2004). Therefore, miRNAs control gene expression by regulating mRNA stability and translation (Eulalio et al. 2008).

Identification of miRNAs

MicroRNAs can be identified either by bioinformatics tools, a computational method or by using experimental approaches.

Bioinformatics Tools of miRNAs Prediction

Several researchers have utilized computational approaches to predict miRNAs based on conserved sequence characteristics across the species. These approaches rely on a combination of RNA secondary structure analyses and conservation of the miRNA sequences among related plant genomes. Based on the sequence conservation and structure homologies of the genome across different species, it has paved the way to discover miRNAs in different organisms by using various bioinformatic tools with the help of known miRNAs of model plants such as Arabidopsis and *O. sativa*. Every day the number of computational methods for the identification of plant miRNAs is increasing. As the plant miRNAs regulate their target mRNAs based on sequence complementarity, it is also possible to predict the target genes in plants. There are many bioinformatic tools available for target gene prediction. Computational tools are also available for the prediction of secondary structure of precursor miRNAs (pre-miRNA). These computer based programs are highly efficient in identifying the miRNAs in plant species with the help of the data of experimentally validated miRNAs. These programs trained or validated using miRNA sequence and targets. Table 2.1 listed the bioinformatics tools for miRNA and target predication and miRNA database.

miRNA Prediction Tools

There are numerous computational methods implemented for miRNA gene prediction based on sequence conservation and/or structural similarity. A program for identification of miRNAs, called MiRscan was developed with 70% specificity at a sensitivity of 50% (Lim et al. 2003). MiRscan program utilized various miRNA features with associated weights to build a bioinformatic tool, which assigns scores to hairpin candidates. Many other researchers employed homology searches for revealing paralog and ortholog miRNAs (Weber 2005). Additionally, Wang and others (Wang et al. 2005) designed a program based on sequence and structure alignment for miRNA prediction. Another program, ProMiR (Nam et al. 2005) is based on machine learning for miRNA discovery that uses a highly specific probabilistic model (HMM) whose topology and states are handcrafted based on prior understanding and assumptions, and accumulated data is used to derive exact probabilities. RNAmicro (Hertel and Stadler 2006) is another miRNA prediction tool developed by Hertel and Stadler is based on comparative sequence analysis instead of structural features. MiRank (Xue et al. 2005) is a novel ranking algorithm based on a random walk through a graph consisting of known miRNA examples and unknown candidate sequences. There are many such tools to predict miRNAs in different plant species based on the data obtained from model plants. Some of the important programs are listed in Table 2.1.

Table 2.1 List of bioinformatics tools for miRNAs prediction, identification and characterization

miRNA prediction tools	
MiRseeker	
MiRscan	http://genes.mit.edu/mirscan/
miRank	MiRank is programmed in Matlab
ProMiR II	http://cbit.snu.ac.kr/~ProMiR2/
PalGrade	
mir-abela	http://www.mirz.unibas.ch/cgi/pred_miRNA_genes.cgi
triplet-SVM	http://bioinfo.au.tsinghua.edu.cn/mirnasvm/
Vmir	http://www.hpi-hamburg.de/fileadmin/downloads/VMir.zip
RNA micro	http://www.bioinf.uni-leipzig.de/~jana/software/index.html
mirCoS	Based on LIBSVM library package
BayesMiRNAfind	https://bioinfo.wistar.upenn.edu/miRNA/miRNA/login.php
One-ClassMirnaFind	http://wotan.wistar.upenn.edu/OneClassmiRNA/
miRFinder	http://www.bioinformatics.org/mirfinder/
Mireval	http://tagc.univ-mrs.fr/mireval
Target prediction tools	
TargetScanS	http://genes.mit.edu/targetscan
miRanda	http://www.microma.org
PicTar	http://pictar.bio.nyu.edu
RNAhybrid	http://bibiserv.techfak.uni-bielefeld.de/rnahybrid
Diana-microT	http://www.diana.pcbi.upenn.edu/cgi-bin/micro_t.cgi
Target Boost	https://demo1.interagon.com/demo
Rna22	http://cbcsrv.watson.ibm.com/rna22_targets.html
MicroTar	http://tiger.dbs.nus.edu.sg/microtar/
NbmiRTar	http://wotan.wistar.upenn.edu/NBmiRTar
miRTour	http://bio2server.bioinfo.uni-plovdiv.bg/miRTour/
miRecords	http://mirecords.umn.edu/miRecords/
miRU	http://bioinfo3.noble.org/miRNA/miRU.htm
TAPIR	http://bioinformatics.psb.ugent.be/webtools/tapir
Target-align	http//www.leonxie.com/targetAlign.php
miTarget	http://cbit.snu.ac.kr/~miTarget
microRNA.org	http://www.microrna.org/microrna/home.do
mirWIP	http://146.189.76.171/query.php
MicroCosm Targets	http://www.ebi.ac.uk/enright-srv/microcosm/htdocs/targets/v5/
miRNA database	
MiRBase	http://microrna.sanger.ac.uk/
TarBase	http://diana.cslab.ece.ntua.gr/tarbase/
Argonaute	http://www.ma.uni-heidelberg.de/apps/zmf/argonaute/
miRecords	http://mirecords.umn.edu/miRecords/
‘miRNAMap 2.0	http://mirnamap.mbc.nctu.edu.tw/
PMRD	http://bioinformatics.cau.edu.cn/PMRD
CSRDB	http://sundarlab.ucdavis.edu/smrnas/
deepBase	http://deepbase.sysu.edu.cn/
miRNA secondary structure prediction tools	
RNA mFold	http://rna.tbi.univie.ac.at/cgi-bin/RNAfold.cgi
miRNA deep sequencing tools	
mirTools	http://59.79.168.90/mirtools/

(continued)

Table 2.1 (continued)

miRDeep	http://www.mdc-berlin.de/en/research/research_teams/systems_biology_of_gene_regulatory_elements/projects/miRDeep/index.html
deepBase	http://deepbase.sysu.edu.cn/
miRExpress	http://mirexpress.mbc.nctu.edu.tw/

Databases of miRNA/Gene Targets

There are very useful databases in public or private domain that provide a significant amount of information on miRNA and target gene predictions. The most extensive online database for both miRNA and target sequences is miRBase that contains both miRNA mature sequences, hairpin sequences of precursors and associated annotation. Release 18.0 of the database contains 18,226 hairpin precursor miRNA, expressing 21,643 mature miRNA products, and 1,929 novel mature miRNAS in 168 species including plants. MiRBase provides miRNA sequence data, annotation, references in the miRBase::Sequences, provides a gene naming and nomenclature facility in the miRBase:: Registry and contains predicted miRNA target genes in miRBase::Targets. TarBase contains a set of experimentally supported targets in different species that are collected manually from the literature. TarBase version 5 includes more than 1,300 experimentally validated miRNA target interactions. The database has information about the target site described by the duplex of miRNA and gene. The database also provides information on the experiments that were executed to validate the target genes, the efficacy of the site to induce translational repression or degradation of mRNA, and a complete reference to the article for in depth understanding.

PMRD (plant microRNA database) database includes all publicly known plant miRNA sequences including those in miRBase. It contains sequence information, secondary structure, target genes, expression profiles and a genome browser. The information is available for 121 plant species including model plants and major crops such as Arabidopsis, rice, wheat, soybean, maize, sorghum, barley, etc. Also for some crops like Arabidopsis, rice, poplar, soybean, cotton, medicago and maize the target genes for miRNAs with a predicted interaction site is available in the database. CSRDB (Cereal Small RNA Database), a database consisting of large scale datasets of maize and rice of small RNA sequences generated by high-throughput pyrosequencing. Small RNA sequences have been mapped to the rice genome and to the available maize genome sequence using the Generic Genome Browser.

miRNA Target Prediction

Recently developed bioinformatics tools have integrated analysis of expression profiles of microRNA and mRNA in conjunction with the predicted microRNA targets in order to minimize false positives and to detect the functional microRNA targets under a specific biological condition. Most of such computational tools rely on the simple principle that inverse relationships in the expression profiles of miRNAs and mRNAs should be held between a specific miRNA and its mRNA targets.

It should be noted that these tools are only reliable for the mRNA targets which are regulated by miRNA mediated degradation, and not sensitive to the targets that are regulated by microRNA mediated translational inhibition.

miRU is a potential plant mRNA target finder. Using this database, potential targets of miRNAs can be predicted. The mature miRNA sequence of interest has to be submitted to search potential targets in cDNA libraries hosted on the miRU server. Its updated version is psRNATarget, a Plant small RNA target analysis server (http://plantgrn.noble.org/psRNATarget/). RNAhybrid, using this database miRNA target prediction can be done. The features are disallowing of G:U base pairs in the seed region at nucleotides 12–18. Minimum free energy can also be calculated by submitting the mature miRNA sequence. miRTour, a Plant miRNA and target prediction tool. This database is mainly used for the detection of plant miRNA and their targets from sequencing datasets (EST, GSS, SRA, etc.). This database automates all the steps of miRNA similarity search, miRNA precursor selection, target prediction and annotation. Each of them can be performed with the same set of input sequences.

TAPIR provides a new feature for predicting novel interactions called 'target mimicry' between miRNAs and their imitated targets. This server is mainly used for the prediction of plant microRNA targets, including target mimics. By using a precise algorithm we can search for the plant miRNA target. The precise option is much slower but guarantees to find less perfectly paired miRNA – target duplexes. In addition, the precise option allows the prediction of target mimics, which are characterized by a miRNA – target duplex having a large loop, making them undetectable by traditional tools. Target-align, an alignment tool designed mainly for accurate prediction of miRNA targets. A score matrix can be build based on the complementarity of nucleotides in order to trace out the optimal local alignments. Important parameters, such as maximum mismatches and maximum consecutive mismatches between miRNAs and their targets, were also used for filtering the optimal local alignments. Target-align can identify multi-target sites as well potential for non-cleaved targets sites. Target-align integrates numerous factors influencing miRNA–target interactions and allows users to set a variety of parameters including alignment and maximum scores, number of consecutive mismatches, base site restrictions and numbers of G:U wobbles and gaps to refine the predictions

Secondary Structure Prediction

There are many programs for predicting the secondary structure of RNA molecule. Using RNAmFold secondary structures of single stranded RNA can be predicted. It is currently packaged in the Vienna RNA website, a collection of tools for folding, designing and analyzing of RNA sequences. This also provides additional analysis of folding parts using the barriers program and structural RNA alignments. The bioinformatics package contains basic programs such as RNAFold for structure prediction of single sequences and also for folding the minimum free energy (MFE) secondary structure, RNAalifold for consensus miRNA structure prediction on a set of aligned sequences, RNAinverse for sequence design, RNAcofold and RNAup for RNA-RNA interaction analysis, LocARNA for the generation of structural alignment and barriers.

Experimental Tools of miRNAs Identification

MicroRNAs can be identified experimentally using five approaches: genetic screening (Lee et al. 1993; Wightman et al. 1993), direct cloning after isolation of small RNAs (Lu et al. 2005a), expressed sequence tags (ESTs) analysis (Zhang et al. 2005), hybridization based approaches and next generation deep sequencing tools.

Genetic Screening

It was the classical approach to discover miRNAs role in gene regulation. In the year 1993, it was found that a small RNA (22 nt) derived from an endogenous gene called *lin-4* suppressed the expression of *lin-14*, which controls the timing of *C. elegans* larval development (Lee et al. 1993; Wightman et al. 1993). Method for identifying miRNAs by genetic screening was similar to methods for identifying other traditional genes through mutational analysis. The application of this method is limited because it is expensive, time consuming, and dominated by chance.

Direct Cloning and Sequencing

To overcome the limitations of genetic screening, approach involving direct cloning after isolation and purification of small RNAs (Fu et al. 2005; Lu et al. 2005a) was discovered. This approach requires isolation of small RNA molecules by followed by ligation of small RNAs to RNA adapters at their 5′ and 3′ ends. Finally, they are reverse transcribed into cDNA using reverse transcription, which is then amplified and sequenced (Lu et al. 2005a). Direct cloning is a more effective method to obtain miRNAs than general genetic screening as only small RNAs are isolated and screened by this method. This strategy was applied to identify stress associated miRNA of Arabidopsis seedlings (Sunkar and Zhu 2004). The big challenge with this approach is to screen the small RNA sequences and distinguish miRNAs from siRNAs and many of the small RNAs involved in different metabolic processes. Novel small RNAs identified by cloning approaches need to be annotated as putative miRNAs using experimental and computational filters (Ambros et al. 2003).

Expressed Sequence Tag (EST) Analysis

miRNAs are evolutionarily conserved from species to species. This feature is extremely useful in prediction of homologous or orthologues of previously known miRNAs. 481 homologues of miRNAs were identified based on previously known miRNAs of Arabidopsis. However, this method can be used to identify only conserved miRNAs (Zhang et al. 2005). The approach based on EST sequences can be well utilized as an attractive and complementary method of miRNA identification.

This approach is particularly useful in the cases where genome sequences are not known. The miRNAs which are species specific and are non-conserved, it is impossible to find these genes based on EST approach.

Microarray-Based Approaches

Various laboratories established that miRNA microarrays succeeded in evaluating miRNA expression on a global scale and empowered the expression profiling of hundreds of miRNA genes together. miRNA arrays are now being developed to explore the biogenesis of miRNAs, tissue distribution, differential miRNA expression between treated and non-treated tissues of plants. The microarrays use a high-density probe either synthetic oligonucleotides or cDNA fragments as capture probes set that can cover nearly every nucleotide in a genome or portion of a genome (Kapranov et al. 2002; Yamada et al. 2003). An ideal probe should have high specificity and high affinity. The microarray approach of expression analysis offers the potential to identify novel transcripts, including miRNA precursors. This approach has been successfully used in Arabidopsis for identification of polyadenylated RNAs from unannotated regions of the genome (Yamada et al. 2003). Several probe and array designs have been described specifically for the detection of known miRNAs using microarrays. In addition to probes that are complementary to the sense and antisense strands of miRNAs, different control probes are also required. They include exogenous and endogenous positive controls and negative controls. The miRNA microarrays allow for the detection of specific sequences, and can be used to assess miRNA differential expression between different tissues, growth stages, treatments or genotypes (Axtell and Bartel 2005; Kapranov et al. 2002; Yamada et al. 2003) and to analyze the expression profile of the corresponding targets genes. MicroRNA arrays can identify the expression of several 100 genes in the same sample at once while requiring only small amounts of total RNA. However, it is unlikely that the current microarray technologies will suffice the requirement for de novo identification of miRNAs, because of low signal owing to the low expression level of most endogenous miRNAs.

The Next Generation Sequencing (NGS) Approaches

The power of NGS technologies for miRNA identification and characterization has been particularly remarkable. The advent of high throughput sequencing methodologies has provided unprecedented opportunities to generate comprehensive sequencing data for the identification and quantification of known and novel miRNAs. These technological leaps forward pose new challenges for the biological interpretation of large sequencing data sets. With the help of NGS tools, miRNA family members, precursors as well as miRNA modifications can be easily identified. This approach of miRNA discovery involves the application of massively parallel signature sequencing (MPSS) to miRNA sequencing. The whole process is completed

in various steps such as size fractionation of total RNA to get small RNAs, adaptor ligation and reverse transcription, size selection and sequencing. These sequencing methods generates high coverage of sequence and sets of more than 350,000 individual sequences per tissue or treatment representing substantial advance over existing methods for the identification of these RNA molecules. The expression profiling of miRNA can be performed by assessing the relative abundance of distinct sequences in each library. The small RNA MPSS data revealed that among the most abundant small RNAs are many miRNAs. Using this approach 77 of 92 available Arabidopsis miRNAs in the miRNA database identified (Lu et al. 2005b). This study was the first claim of a parallel, high-throughput sequencing methods for the identification and characterization of miRNAs.

There are many new high throughput sequencing approaches and chemistries that have recently been developed, and these are likely to deliver novel means of miRNA discovery. Two new sequencing technologies were introduced based on sequencing by synthesis (SBS). The 454 Life Sciences (http://www.454.com/), using pyrosequencing technology enables high-throughput, parallel sequencing of hundreds of thousands of DNA or cDNA fragments (Margulies et al. 2005). One major advantage of this technology is the longer read length of about 1 Kb that allows the full-length sequencing of miRNAs and its precursor. The other system Solexa http://www.illumina.com website, detects fluorescence signals that promises to generate tens of millions of 25-nt tags, potentially offering the richest source of small RNA data. Both the technologies execute millions of sequencing reactions in parallel, producing data at ultrahigh rates. In the past year, Applied Biosystems has introduced their SOLiD sequencer http://www3.appliedbiosystems.com webcite, another short-read 20–35 bp platform, with read lengths anticipated to be 50 bp in the upcoming SOLiD3 release. Similarly, miRNAs can be identified using Illumina platform also that offers good coverage and reasonable good read length. These sequencing platforms offer a variety of experimental approaches for characterizing a transcriptome, including single-end and paired-end cDNA sequencing, tag profiling, methylation assays, secondary structure analysis, miRNA sequencing, alternate polyadenylation, fusion gene analysis and splice variant analyses. These technologies offer advantages in both cost and throughput of several orders of magnitude over traditional sequencing methods. This would presumably require modifications for the sequencing of small RNAs, but this would appear to be straightforward based on the previous success with MPSS. The deep sequencing technologies have the advantage over microarray based approaches that novel sequences can be detected while microarrays can only identify expression profile of known miRNAs. Further, next generation sequencing technologies are highly sensitive and dynamic range can be gained by the high sequencing depth. Next generation sequencing is independent of predesigned probes, thus making it very suitable for the discovery of new miRNAs. Nevertheless, deep sequencing is a relatively novel approach and the associated computational analysis tools are still in their infancy and need to be improved to standardize normalization, mapping and thresholding (Motameny et al. 2010).

Role of miRNAs in Plant Metabolism

MicroRNA are said to be involved in various metabolic processes in plants like growth and development, morphogenesis, flowering, stress responses (biotic and abiotic stresses) (Khraiwesh et al. 2012), signal transduction (Meng et al. 2010; Zhang et al. 2006) and in feedback regulation of genes (Meng et al. 2010; Yanga et al. 2007). The miRNAs have been identified in many plant species like Arabidopsis, brassica, rice, wheat, barley etc. In various experiments, it has been demonstrated that miRNAs regulate various plant development processes, such as leaf morphogenesis and polarity (Kim et al. 2005), floral differentiation (Chen 2004), root initiation (Guo et al. 2005), vascular development (Kim et al. 2005), and transition of plant growth from vegetative growth to reproductive growth (Lauter et al. 2005). A majority of these miRNAs regulate expression of transcription factors that influence cell fate determination and ultimately affect plant traits (Mallory et al. 2004; Rhoades et al. 2002). Interestingly, about half of the identified miRNA targets are transcription factors involved in regulation of key metabolic processes of plants (Bartel 2004). Of these miRNAs, a great number of miRNA families target genes encoding transcriptional factors that regulate plant development (Allen et al. 2004). A number of studies demonstrated that a majority of miRNAs regulate plant development by controlling the levels of transcription factors.

MicroRNAs regulate key components of hormone signaling pathways also and further regulate hormone homeostasis and related developmental processes (Guo et al. 2005; Mallory et al. 2005). Several miRNAs (miR159, miR160, miR164, and miR167) were characterized from plant tissues induced by abscisic acid (ABA), Gibberellic acid (GA), jasmonic acid (JA), salicylic acid (SA), and other phytohormones (Zhang et al. 2005). In addition to miR160, miR164, and miR167, other miRNAs, such as miR393, are probably involved in signaling pathways by regulating TIR1 which is an important component of a SCF E3 ubiquitin ligase that degrades Aux/IAA proteins in response to auxin (Gray et al. 2001; Mallory et al. 2005). This study is important as demonstrates that miRNA can also regulate F-box proteins and affect the activity of the E3 enzyme. In conclusion, studies indicate that miRNAs are involved in plant metabolism through hormone-mediated signal transduction.

MicroRNA not only regulates the expression of genes controlling plant development but also regulates its own biogenesis and/or function. As of new, at least five miRNAs (miR162, miR168, miR173, miR390, and miR398) are known to regulate miRNA biogenesis or function. The mRNA encoding DCL1 is the target of miR162 (Xie et al. 2003). Similarly, miR168 guided cleavage of AGO1 mRNA is crucial for the post-transcriptional gene regulation of the ago1 transcript (Vazquez et al. 2004). The alteration in the nucleotides of AGO1 mRNA to make it less complementary with miR168 resulted in increasing levels of AGO1 mRNA and consequently caused developmental defects (Vaucheret et al. 2004).

In rice, the deep sequencing of developmental rice grains revealed the involvement of 20 different miRNA families which are expressed in organ specific manner (Zhu et al. 2008). Another study in rice, applying Massively Parallel Signature

Sequencing (MPSS) and integrative analysis identified 26 novel miRNA's and 12 miRNA candidates in rice seeds of which most of the identified miRNA's shown tissue specific expression (Sunkar et al. 2005). In maize also miRNA are detected in the early stages of the developmental processes. Using Solexa technology in maize, 106 novel miRNA has been identified (Wang et al. 2011). miRNA are also said to be in the development of roots and root architecture (Boualem et al. 2008) where auxin pathways plays an important role as shown in rice and Arabidopsis (Meng et al. 2010). The study in rice auxin resistant mutant discovered miRNA signal transduction pathway and also showed the feedback circuit between miRNA and auxin response factors (ARF). 133 miRNA has proved to be expressed tissue specifically and root zone specifically in Arabidopsis (Breakfield et al. 2011). Three miRNA's viz. miR482, miR1512, miR1515 were established for their function in root nodulation as identified in soybean (Li et al. 2010). miRNAs are also involved in meristem identity. Mutants defective in CARPEL FACTORY or Hen1, fail to produce miRNA and thus resulting in several developmental defects (Jones 2002). Zhao et al. using high through put sequencing technology, identified expression differences in miRNA between inferior and superior spikelets in rice. This study revealed the involvement of 43 novel miRNA's which are accumulated differentially in inferior and superior spikelets that are slow grain filling and low grain weight in rice (Peng et al. 2011).

MicroRNAs Involved in Stress Responses

Plants are sessile organisms that must endure stressful environments. Recently, there has been strong evidence leading to the proposal that miRNAs are hypersensitive to abiotic or biotic stress as well as to diverse physiological processes (Lu et al. 2005a; Sunkar and Zhu 2004). Drought, cold, and salinity are major abiotic stresses for plants; all of these conditions strongly induced miR402 over-expression. Other miRNAs, such as miR319, are induced by either cold or other stress (Sunkar and Zhu 2004). The first direct report linking miRNA and stress tolerance was miR398, expression of which is transcriptionally down-regulated by oxidative stresses. In Arabidopsis, miR398 was found to target two closely related Cu/Zn superoxide dismutase coding genes, cytosolic CSD1 and chloroplastic CSD2, and a reduced level of miR398 led to improved tolerance of transgenic lines compared with the wild-type plants under oxidative stress conditions (Sunkar et al. 2006). In rice, miR169g was showed as the only member induced by drought stress among the miR169 family (Zhao et al. 2007). Furthermore, to determine the role of miRNAs in stress response, 21 miRNAs of Arabidopsis were predicted to be associated under UV-B stress condition (Zhou et al. 2007). In a study using EST analyses, 25.8% of ESTs containing miRNAs were found in stress-induced plant tissues (Zhang et al. 2005). At low-sulfur conditions, the ATP sulfurylase APS4 and the sulfate transporter AST68 are accumulated and both of these genes are regulated by miR395 (Allen et al. 2005; Jones-Rhoades and Bartel 2004). Lu et al. (2005a) identified 48

miRNA sequences from the Populus genome. Majority of these Populus miRNAs showed their target as developmental and stress/defense related genes.

A recent study has identified the involvement of miRNA in relation to submergence tolerance (Zhang et al. 2008). In Arabidopsis, miR399 over expression resulted in the down regulation of the target mRNA transcript, thus, deciphering the role of miRNA to cope up the mineral nutrition fluctuations (Fujii et al. 2005). The study on the dehydration stress on barley identified 28 new miRNA's belonging to 18 families of which five miRNA have experimentally validated for their differential expression (Melda et al. 2010). Similarly, 21 maize miRNA are identified in drought stress of which 13 are proved to be specific for drought stress (Zhang et al. 2010). Many studies discovered numerous rice miRNA involved in different abiotic stresses viz., salinity (Zhao et al. 2009), cold (Lv et al. 2010), heavy metal (Ding et al. 2011; Huang et al. 2009a,), hydrogen peroxide (Li et al. 2011b), ABA (Liu et al. 2009) and drought (Jian et al. 2010; Zhao et al. 2007; Zhou et al. 2010).

MicroRNAs Expression Under Salt Stress

Salt stress is one of the most serious abiotic stresses of crop plants worldwide (Monteiro et al. 2011). Plant salt stress is a condition where excessive salts in soil solution cause inhibition of plant growth or plant death. High salt stress leads to disruption of homeostasis in water potential and ion distribution which occurs at both the cellular and the whole plant levels. Drastic changes in ion and water homeostasis lead to molecular damage, growth arrest and even death of plants (Zhu 2001). Salt stress furthermore induces the ABA synthesis which closes stomata when transported to guard cells that ultimately decreases photosynthesis activity and leads to oxidative stress.

To tolerate the high salt stress in their sessile lifestyle, plants have evolved a considerable degree of developmental plasticity, including adaptation via networks of molecular events. Numerous genes and gene products in plants are affected due to salt stress (Zhu 2002). Large number of gene transcripts gets up or down regulated during salt stress suggesting the tight regulation of transcription during stressed condition in plants. Therefore, post-transcriptional gene regulation plays a crucial role in the plant salt response (Fig. 2.1). MicroRNAs are now known as ubiquitous regulators of gene expression in eukaryotic organisms. In plants, functional analysis has demonstrated that several miRNAs play vital roles in plant resistance to abiotic and biotic stress (Navarro et al. 2006; Sunkar and Zhu 2004). Various studies on Arabidopsis, rice and other plants have revealed an important role for miRNAs in drought and salt responses. Recently in Arabidopsis, several differentially regulated miRNAs have been identified in salt-stressed tissues. In response to salt stress, miR156, miR158, miR159, miR165, miR167, miR168, miR169, miR171, miR319, miR393, miR394, miR396, and miR397 were up-regulated, while the miR398 was down-regulated in Arabidopsis, thus establishing a role for miRNAs in the adaptive response to salt stress (Liu et al. 2008).

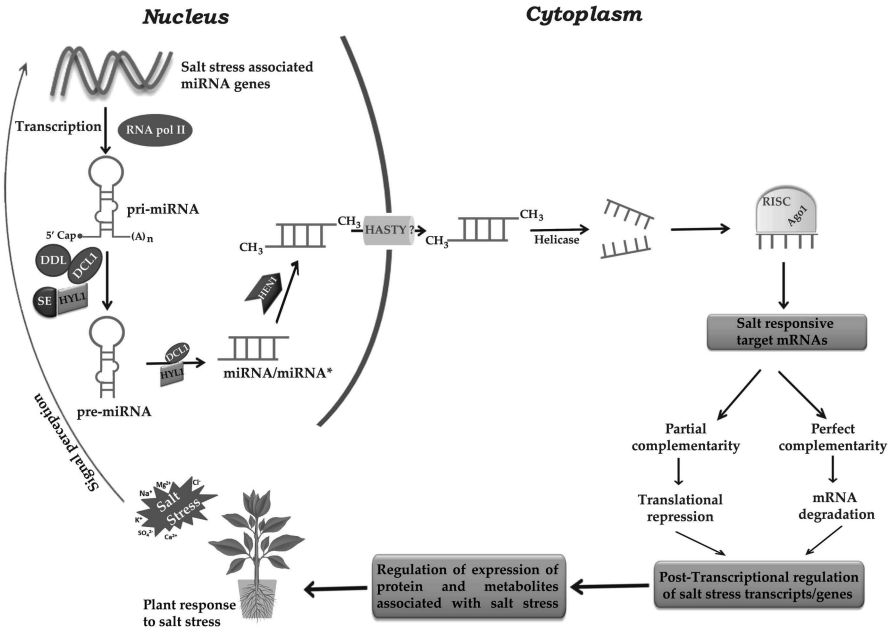

Fig. 2.1 A pathway showing miRNA mediated post-transcriptional regulation of salt stress responsive plant genes

Up-regulation of miRS1 and miR159.2 in response to salt stress was observed in *Phaseolous vulgaris*, (Arenas-Huertero et al. 2009). The expression of miR530a, miR1445, miR1446a-e, miR1447, and miR171l-n was increased, whereas miR482.2 and miR1450 was decreased during salt stress in *P. trichocarpa*, (Lu et al. 2008). Further, two members of miR169 family viz. miR169g and miR169n showed enhanced expression during salinity. Interestingly, a cis-acting ABA-responsive element (ABRE) was identified in the upstream region of miR169n, which gave an indication that miR169n may be regulated by stress hormone ABA. Both miR169g and miR169n showed its target mRNA as Nuclear factor Y subunit A (NF-YA) which was shown to be down-regulated in drought-affected leaves of wheat (Stephenson et al. 2007). Recently, the comparative study between salt tolerant maize genotype NC286 and salt sensitive maize genotypes Huangzao4 demonstrated that miR156, miR164, miR167, and miR396 families were down-regulated, while miR162, miR168, miR395, and miR474 families were up-regulated in salt-stressed maize roots. The study also proposed a gene model that regulates the abiotic stresses and gene networks coping with the stress (Ding et al. 2009). A microRNA miR393 is a conservative miRNA family present in a variety of different plant species. The two members of this miR393 family in rice are osa-MIR393 and osa-MIR393b. The expression level of osa-MIR393 altered, whereas that of osa-MIR393b did not alter under salinity and alkaline stress suggesting the precise regulation of

salt associated genes. Soybean miRNAs associated with salt stress responses have been identified and analyzed by utilizing the next generation sequencing technology and bioinformatics tools. One hundred and thirty-three conserved miRNAs representing 95 miRNA families were differentially expressed in soybeans under different stress treatments along with 50 miRNAs differently expressed under salt stress (Li et al. 2011a). miR159 and 319 were up-regulated following saline treatment in artichoke tissues (De Paola et al. 2012). With the advancement of genomics tools and methods to identify novel miRNAs in various plant species, the number of miRNAs associated with salt stress response is increasing (Table 2.2). A better understanding of miRNA mediated gene regulation under salt stress will certainly help in elucidating the complex network of regulatory molecules, genes, proteins and metabolites.

Regulation of Target Genes of Salt Stress Associated miRNAs

Most miRNAs target multiple targets belonging to the same gene family in plants. Emerging data showed that miRNAs under specific conditions can selectively regulate the expression of specific target genes (Sunkar et al. 2012). Few times about target gene regulation via miRNAs detected in the maize salt stress response showed their target as many transcription factors (TFs) involved in plant development and organ formation which is in close agreement with previously reported data in model plants. The TFs Myb, NAC1 and homeodomain-leucine zipper protein (HD-ZIP) were predicted as the targets of zma-miR159a/b, zma-miR164a/b/c/d and zma-miR166l/m, respectively. The similar reports were made in arabidopsis and rice (Jones-Rhoades and Bartel 2004). Other transcription factors predicted as the miRNA targets include MADS-box proteins and zinc-finger proteins which have been reported as salt stress-responsive factors in plants (Fang et al. 2006; Xu et al. 2008). In addition to transcription factors, several miRNAs target genes that encode proteins in diverse metabolic pathways or are involved in various physiological processes of plants having important function. Many of the predicted targets of miRNAs, such as NADP-dependent malic enzyme (NADP-ME) and cytochrome oxidase, are known as salt stress-responsive plant genes (Cheng and Long 2007; Yan et al. 2005).

The targets sulfurylase and ASP1 genes are regulated by miR395 in salt induced soybean line under sulfate starvation conditions. Therefore the role of miR395 may be in non-specific salt stress responding pathways, such as the maintenance of energy supply (Ding et al. 2009; Jones-Rhoades and Bartel 2004). The targets F-box proteins and a basic-helix-loop-helix family protein induced during salt stress are regulated by miR393a (Jones-Rhoades and Bartel 2004). These stress responsive targets of miRNAs are regulated post-transcriptionally during stress responsive processes. Two putative targets for artichoke, cca-miR397 and 399 were homologous to members of laccase gene family, which has been demonstrated to be involved in salt stress response (De Paola et al. 2012). Laccases are

Table 2.2 List of reported miRNAs and their targets associated with salt stress in plants

S.No	miRNA	Plant species	Sequence	Predicted targets	References
1	miR156	*Arabidopsis thaliana*	UGACAGAAGAGAGUGAGCAC	Squamosa promoter-binding protein-like 11	Liu et al. (2008)
2	miR156	*Zea mays*	UGACAGAAGAGAGUGAGCAC	SBP-domain protein	Liu et al. (2008)
3	miR158	*Arabidopsis thaliana*	UCCCAAAUGUAGACAAAGCA	F-box family protein Pentatricopeptide (PPR) repeat-containing protein	Liu et al. (2008)
4	miR159	*Arabidopsis thaliana*	UUUGGAUUGAAGGGAGCUCUA	MYB and TCP transcription factors	Liu et al. (2008)
5	miR162	*Zea mays*	UCGAUAAACCUCUGCAUCCA	RNAseIII CAF protein Endoribonuclease Dicer Cytochrome P450	Ding et al. (2009)
6	miR164	*Zea mays*	UGGAGAAGCAGGGCACGUGCA	NAC domain protein NAC1	Ding et al. (2009)
7	miR165	*Arabidopsis thaliana*	UCGGACCAGGCUUCAUCCCCC	Class III HD-ZIP transcription factors	Liu et al. (2008)
8	miR166	*Zea mays*	UCGGACCAGGCUUCAUUCCCC	Homeo domain leucine Zipper protein (HD-ZIP)	Ding et al. (2009)
9	miR166b	*Glycine max*	UCGGACCAGGCUUCAUUCCCC	DNA binding protein Class III HD-Zip protein 4 and 8	Li et al. (2011b)
10	miR167	*Arabidopsis thaliana*	UGAAGCUGCCAGCAUGAUCUA	Auxin response factor 6 and 8	Liu et al. (2008)
11	miR167	*Zea mays*	UGAAGCUGCCAGCAUGAUCUA	Auxin response factor	Ding et al. (2009)
12	miR168	*Arabidopsis thaliana*	UCGCUUGGUGCAGGUCGGGAA	AGO1 (ARGONAUTE 1)	Liu et al. (2008)
13	miR168	*Zea mays*	UCGCUUGGUGCAGAUCGGGAC	PZE40 protein, Cytoplasmic aldolase, AGO1-1	Ding et al. (2009)
14	miR169	*Arabidopsis thaliana*	CAGCCAAGGAUGACUUGCCGA	CCAAT-binding transcription factor (CBF-B/NF-YA) family protein	Liu et al. (2008)
15	miR171	*Arabidopsis thaliana*	UGAUUGAGCCGCGCCAAUAUC	Scarecrow transcription factor family protein	Liu et al. (2008)
16	miR172	*Zea mays*	AGAAUCUUGAUGAUGCUGCA	Gamma-tubulin	Ding et al. (2009)
17	miR319	*Arabidopsis thaliana*	UUGGACUGAAGGGAGCUCCCU	TCP transcription factors	Liu et al. (2008)
18	miR393	*Arabidopsis thaliana*	UCCAAAGGGAUCGCAUUGAUCC	F-box protein; bHLH (basic helix–loop–helix) transcription factor	Liu et al. (2008)

19	miR393	*oryza sativa*	UCCAAAGGGAUCGCAUUGAUC	Phytosulfokine receptor precursor, Transport inhibitor response 1 protein Oxidoreductase,	
20	miR394	*Arabidopsis thaliana*	UUGGCAUUCUGUCCACCUCC	F-box family protein	Liu et al. (2008)
21	miR395	*Zea mays*	GUGAAGUGUUUGGGGGAACUC	ATP sulfurylase, L-Isoaspartyl methyltransferase, Beta-D-xylosidase, 8 NADP-dependent malic protein	Ding et al. (2009)
22	miR395	*Glycine max*	AUGAAGUGUUUGGGGGAACUC	Dehydration-responsive element binding protein 6 A7X2S6 sulfate transporter ATP sulfurylase Beta-glucosidase Disease resistance protein Glycoside hydrolase, family 17; Virulence factor, pectin lyase fold; Beta-glucosidase Phosphoadenylylsulfate reductase Dopamine beta-monooxygenase	Li et al. (2011b)
23	miR396	*Arabidopsis thaliana*	UUCCACAGCUUUCUUGAACUG	GRF2 transcription factor Rhodenase-like protein; Kinesin-like protein B	Liu et al. (2008)
24	miR396	*Zea mays*	UUCCACAGCUUUCUUGAACUG	Cytochrome oxidase subunit I	Ding et al. (2009)

(continued)

Table 2.2 (continued)

S.No	miRNA	Plant species	Sequence	Predicted targets	References
25	miR396c	*Oryza sativa*	UUCCACAGCUUUCUUGAACUU	heat shock 70 kDa protein 4, TBP-associated 59 kDa subunit protein, Leucine rich repeat family protein Ubiquitin-protein ligase COP1, At GRF 2 and 5 Growth regulating factor1 NBS-LRR type disease resistance protein IQ calmodulin-binding motif family protein Jasmonate O-methyltransferase	Gao et al. (2010)
26	miR397	*Arabidopsis thaliana*	UCAUUGAGUGCAGCGUUGAUG	LAC2 (laccases); ß-6 tubulin	Liu et al. (2008)
27	miR398	*Arabidopsis thaliana*	UGUGUUCUCAGGUCACCCCUU	InterPro domain Protein of unknown function DUF266, plant	Liu et al. (2008)
28	miR417	*Arabidopsis thaliana*	GAAGGUAGUGAAUUUGUUCGA	C2-domain containing protein SNF7family protein, contains Pfam domain Hydrolase Cell expansion protein RNA-directed RNA polymerase SNF domain/helicase domain protein Auxin response transcription factor	Jung and Kang (2007)
29	miR482.2	*Populus trichocarpa*	UCUUGCCUACUCCUCCCAUU	Disease resistance protein	
30	miR530a	*Populus trichocarpa*	UGCAUUUGCACCUGCACCUU	Zinc knuckle (CCHC-type) family protein Homeobox transcription factor KN3 Ribosomal protein L1 family protein	Lu et al. (2008)
31	miR1445	*Populus trichocarpa*	UCCCUUGUAGACUAGAAAAA	Dihydropyrimidinase	Lu et al. (2008)

32	miR1446a-e	*Populus trichocarpa*	UUCUGAACUCUCUCCCUCAA	GCN5-related N-acetyltransferase (GNAT) Family protein Gibberellin response modulator-like protein, Replication factor C-like Homeodomain transcription factor	Lu et al. (2008)
33	miR1447	*Populus trichocarpa*	CAGAAUUGCAGUGCCUUGAUU	Ankyrin repeat family protein, Beta-fructofuranosidase Oxidoreductase Leucine-rich repeat transmembrane protein kinase Disease resistance protein Translationally controlled tumor protein	Lu et al. (2008)
34	miR1450	*Populus trichocarpa*	UUCAAUGGCUCGGUCAGGUUAC	Leucine-rich repeat transmembrane protein kinase	Lu et al. (2008)
35	miR1507a	*Glycine max*	UCUCAUUCCAUACAUCGUCUG	Splicing factor yt521-B, NBS-LRR resistance protein RGH1 Cytosine-specific methyltransferase FAD linked oxidase, N-terminal	Li et al. (2011b)
36	miR 2001	*Oryza sativa*	CCCAGCUUGAGAAUCGGGCGGC	Hypothetical protein Protein GPR107 precursor	Jian et al. (2010)
37	miR2003	*Oryza sativa*	CCGGCCCCGAACCCGUCGGCU	HEAT repeat family protein, expressed Ribosomal protein S11 containing protein, Expressed protein BGGP Beta-1-3-galactosyl-O-glycosylglycoprotein, Hypothetical protein NAC domain-containing protein 90	Jian et al. (2010)

(continued)

Table 2.2 (continued)

S.No	miRNA	Plant species	Sequence	Predicted targets	References
38	miR2004	*Oryza sativa*	GACCGCAUAGCGCAGUGGAUU	EMB2745, Exonuclease, Lectin receptor-type protein kinase, FAD binding domain containing protein, Serine/threonine-protein kinase Hypothetical protein Oxidoreductase, expressed Peroxidase 52	Jian et al. (2010)
39	miR2006	*Oryza sativa*	GUGGCUGUAGUUUAGUGGUGA	Hypothetical protein Conserved hypothetical protein	Jian et al. (2010)

multicopper-containing glycoproteins, present in plants. It has been reported that the expression level of laccase genes is enhanced by high concentrations of NaCl in tomato, maize, and Arabidopsis roots (Cai et al. 2006; Liang et al. 2006; Wei et al. 2000). In artichoke, reduces expression of miR397a in roots during salt stress might possibly lead to enhanced expression of laccase (De Paola et al. 2012). A salt responsive target aspartic proteinase APA1 was predicted to be regulated by miRNA cca-novel-18. In Arabidopsis, a low decrease in the expression of target superoxide dismutase together with a slight increase in miR398 expression was observed under NaCl treatment (Attia et al. 2011). Another important target ARGONAUTE1 (AGO1) gene, which encodes the RNA slicer enzyme in the miRNA pathway, is regulated by miR168 (Wei et al. 2000). miR168 and AGO1 both are crucial in maintaining the balance between miRNAs and their target genes. The miR168 has also been found to be involved in salt stress in maize (Ding et al. 2009).

MicroRNAs Application in Development of Salt Stress Tolerant Plants

Understanding new RNA-guided stress regulatory networks should provide new way for the genetic improvement of plant stress tolerance. Indeed, it has been shown in few reports that manipulation of miRNA-guided gene regulation can help to engineer plants that will be more salt stress-tolerant. Differences in the expression level of miRNAs between inbred and hybrid lines of maize were studied. Under salt stress modest up-regulation of miR156 and miR166 in B73 and Mo17 lines was observed while an almost 2.5-fold up-regulation for miR156 and a 1.8-fold up-regulation for miR166 were observed in Mo17 × B73 hybrid. These results showed that the hybrid lines had drastic change in the miRNA expression and more flexible for salt stress when compared to the parents (Kong et al. 2010). Transgenic rice and *Arabidopsis thaliana* plants constitutively over-expressing osa-MIR396c showed reduced salt and alkali stress tolerance compared to that of wild-type plants (Gao et al. 2010). Similar efforts were made to overexpress osa-MIR393 in transgenic rice and Arabidopsis and it was shown that transgenics were sensitive to salt and alkali treatment as compare to wild-type plants. These results demonstrated that over-expression of osa-MIR393 can regulate rice salt-alkali stress tolerance in negative fashion (Gao et al. 2011). Transgenic Arabidopsis plants constitutively over-expressing miRNA417 showed that seed germination of the transgenic plants was retarded compared with the wild-type plants in the presence of high salt or ABA. These results also indicated that the miRNA417 plays a role of negative regulator of seed germination in Arabidopsis plants under salt stress conditions (Jung and Kang 2007). Recent study showed that specific downregulation of the bacterial-type PEPC (Phosphoenolpyruvate carboxylase) gene, Atppc4, by artificial microRNA improved salt tolerance in Arabidopsis. The improved salt tolerance may be related with the improved PEPC activity (Wang et al. 2012).

Conclusion and Future Perspective

Salinity is a significant problem affecting physiological, biochemical and molecular processes of plants and is predicted to become a larger problem in the coming decades. The detrimental effects of high salinity on plants can be observed at the whole-plant level or in the cellular level in terms of plant death and/or decrease in productivity. One way in which plants respond to salt stress is by modifying their gene expression through the post transcriptional gene regulation. MicroRNAs regulate the post-transcriptional expression of associated target genes involved in salt stress response. Hence, in addition to their roles in growth and development and maintenance of genome integrity, miRNAs are also important components in plant stress responses. Therefore, understanding how miRNAs regulate gene expression will enable researchers to explore the role of miRNAs in salt stress response in different plant species. Computational and experimental approaches have accelerated the efforts to discover large number of miRNAs and their targets in plants associated with various traits. The availability of complete genome information and next generation sequencing technologies has accelerated the efforts to understand the miRNA regulation during various abiotic and biotic stresses.

Identification of entire sets of miRNAs and their targets will lay the foundation that is needed to unravel the complex miRNA mediated regulatory networks controlling development and other physiological processes such as salt stress. The targets of plant miRNAs often belong to families of transcription factors involved in the control of genes associated with a particular trait. Given that miRNAs are crucial components in gene regulatory networks, it is certain that a complete understanding of the functions of miRNAs will greatly increase our understanding of plant tolerance to salt stress. Despite the existing voluminous data relating the miRNAs, a lot more remains to be known in terms of identification and characterization of the unknown miRNAs in diverse systems. Understanding miRNA-guided stress regulatory networks will provide new tools for the genetic improvement of salt tolerance in plants. Although this field is still in its infancy, the idea that miRNAs can be used in the therapy of plant stress is certain. If smart miRNAs can be used appropriately, a new avenue of biotechnology aimed at achieving enhanced salt tolerant plants will be opened. There are few reports showing that manipulation of miRNA guided gene regulation can help in the engineering of stress-resistant plants. Nevertheless, understanding of miRNA evolution is just at the starting point for elucidating their complex regulatory roles.

Studying stress-responsive miRNAs and their target gene expression in particular cell types will provide greater insights into miRNA target networks that operate in a cell- or tissue-specific manner during stress. Information generated from characterization of new small RNAs and the regulatory network of salt stress response needs to be scrutinized for development of tools to enhance plant tolerance to high salinity. It is a challenge to identify and characterize the catalogue of small RNAs that exhibit alteration in their expression level upon salt stress in various crop plants and to discover the target genes of those newly discovered miRNAs. Overcoming this challenge will allow

rapid deciphering of new components in plant stress tolerance and lead to elucidation of the complex regulatory network of salt stress response. As our understanding of the roles of miRNAs during salt stress deepens, the possibilities for using miRNA-mediated gene regulation to enhance plant stress tolerance will become enormous.

Acknowledgments Authors thank Project Director (Directorate of Rice Research) for valuable support and encouragement. B. Sailaja acknowledges funding support and fellowship obtained from the NICRA (National Initiative on Climate Resilient Agriculture) project.

References

Agarwal S, Mohan M, Mangrauthia SK (2011) RNAi: machinery and role in pest and disease management. In: Bandi V, Shanker AK, Shanker C, Mandapaka M (eds) Crop stress and its management: perspectives and strategies. Springer, Netherlands, pp 447–469

Allen E, Xie Z, Gustafson AM, Sung GH, Spatafora JW, Carrington JC (2004) Evolution of microRNA genes by inverted duplication of target gene sequences in *Arabidopsis thaliana*. Nature Genet 36:1282–1290

Allen E, Xie Z, Gustafson AM, Carrington JC (2005) microRNA-directed phasing during trans-acting siRNA biogenesis in plants. Cell 121:207–221

Ambros V, Bartel B, Bartel DP, Burge CB, Carrington JC, Chen X, Dreyfuss G, Eddy SR, Griffiths-Jones S, Marshall M, Matzke M, Ruvkun G, Tuschl T (2003) A uniform system for microRNA annotation. RNA 9:277–279

Arenas-Huertero C, Pérez B, Rabanal F, Blanco-Melo D, De la Rosa C, Estrada-Navarrete G, Sanchez F, Covarrubias A, Reyes J (2009) Conserved and novel miRNAs in the legume *Phaseolus vulgaris* in response to stress. Plant Mol Biol 70:385–401

Attia H, Karray N, Msilini N, Lachaâl M (2011) Effect of salt stress on gene expression of superoxide dismutases and copper chaperone in *Arabidopsis thaliana*. Biologia Plantarum 55:159–163

Axtell MJ, Bartel DP (2005) Antiquity of microRNAs and their targets in land plants. Plant Cell 17:1658–1673

Bartel DP (2004) MicroRNAs: genomics, biogenesis, mechanism, and function. Cell 116:281–297

Bernstein E, Caudy AA, Hammond SM, Hannon GJ (2001) Role for a bidentate ribonuclease in the initiation step of RNA interference. Nature 409:363–366

Boualem A, Laporte P, Jovanovic M, Laffont C, Plet J, Combier JP, Niebel A, Crespi M, Frugier F (2008) MicroRNA166 controls root and nodule development in *Medicago truncatula*. Plant J 54(5):876–887

Breakfield NW, Corcoran DL, Petricka JJ, Shen J, Sae-Seaw J, Rubio-Somoza I, Weigel D, Ohler U, Benfey PH (2012) High-resolution experimental and computational profiling of tissue-specific known and novel miRNAs in Arabidopsis. Genome Res 22(1):163–176

Brodersen P, Voinnet O (2006) The diversity of RNA silencing pathways in plants. Trends Genet 22:268–280

Cai X, Davis EJ, Ballif J, Liang M, Bushman E, Haroldsen V, Torabinejad J, Wu Y (2006) Mutant identification and characterization of the laccase gene family in Arabidopsis. J Exp Bot 57:2563–2569

Chen X (2004) A microRNA as a translational repressor of APETALA2 in Arabidopsis flower development. Science 303:2022–2025

Cheng Y, Long M (2007) A cytosolic NADP-malic enzyme gene from rice (*Oryza sativa* L.) confers salt tolerance in transgenic Arabidopsis. Biotechnol Lett 29:1129–1134

De Paola D, Cattonaro F, Pignone D, Sonnante G (2012) The miRNAome of globe artichoke: conserved and novel micro RNAs and target analysis. BMC Genomics 13:41

Ding D, Zhang L, Wang H, Liu Z, Zhang Z, Zheng Y (2009) Differential expression of miRNAs in response to salt stress in maize roots. Ann Bot 103:29–38

Ding Y, Chen Z, Zhu C (2011) Microarray-based analysis of cadmium-responsive microRNAs in rice (*Oryza sativa*). J Exp Bot 62(10):3563–3573

Dong Z, Han MH, Fedoroff N (2008) The RNA-binding proteins HYL1 and SE promote accurate in vitro processing of pri-miRNA by DCL1. Proc Natl Acad Sci USA 105(29):9970–9975

Du T, Zamore PD (2005) microPrimer: the biogenesis and function of microRNA. Development 132:4645–4652

Eulalio A, Huntzinger E, Izaurralde E (2008) GW182 interaction with Argonaute is essential for miRNA-mediated translational repression and mRNA decay. Nat Struct Mol Biol 15:346–353

Fang Q, Xu Z, Song R (2006) Cloning, characterization and genetic engineering of FLC homolog in *Thellungiella halophila*. Biochem Biophys Res Commun 347:707–714

Floyd SK, Bowman JL (2004) Gene regulation: ancient microRNA target sequences in plants. Nature 428:485–486

Fu H, Tie Y, Xu C, Zhang Z, Zhu J, Shi Y, Jiang H, Sun Z, Zheng X (2005) Identification of human fetal liver miRNAs by a novel method. FEBS Lett 579:3849–3854

Fujii H, Chiou TJ, Lin SI, Aung K, Zhu JK (2005) A miRNA involved in phosphate-starvation response in Arabidopsis. Curr Biol 15(22):2038–2043

Gao P, Bai X, Yang L, Lv D, Li Y, Cai H, Ji W, Guo D, Zhu Y (2010) Over-expression of osa-MIR396c decreases salt and alkali stress tolerance. Planta 231:991–1001

Gao P, Bai X, Yang L, Lv D, Pan X, Li Y, Cai H, Ji W, Chen Q, Zhu Y (2011) osa-MIR393: a salinity- and alkaline stress-related microRNA gene. Mol Biol Rep 38:237–242

Gray WM, Kepinski S, Rouse D, Leyser O, Estelle M (2001) Auxin regulates SCF (TIR1)-dependent degradation of AUX/IAA proteins. Nature 414:271–276

Guo HS, Xie Q, Fei JF, Chua NH (2005) MicroRNA directs mRNA cleavage of the transcription factor NAC1 to downregulate auxin signals for Arabidopsis lateral root development. Plant Cell 17:1376–1386

Hasegawa PM, Bressan RA, Zhu JK, Bohnert HJ (2000) Plant cellular and molecular responses to high salinity. Annu Rev Plant Physiol Plant Mol Biol 51:463–499

Hertel J, Stadler PF (2006) Hairpins in a Haystack: recognizing microRNA precursors in comparative genomics data. Bioinformatics 22(14):e197–e202

Hu Y, Burucs Z, von Tucher S, Schmidhalter U (2007) Short-term effects of drought and salinity on mineral nutrient distribution along growing leaves of maize seedlings. Environ Exp Bot 60(2):268–275

Huang SQ, Peng J, Qiu CX, Yang ZM (2009a) Heavy metal-regulated new microRNAs from rice. J Inorg Biochem 103(2):282–287

Huang Y, Ji L, Huang Q, Vassylyev DG, Chen X, Ma JB (2009b) Structural insights into mechanisms of the small RNA methyltransferase HEN1. Nature 461:823–827

Jian X, Zhang L, Li G, Zhang L, Wang X, Cao X, Fang X, Chen F (2010) Identification of novel stress-regulated microRNAs from *Oryza sativa* L. Genomics 95(1):47–55

Jones L (2002) Revealing micro-RNAs in plants. Trends Plant Sci 7(11):473–475

Jones-Rhoades MW, Bartel DP (2004) Computational identification of plant micro- RNAs and their targets, including a stress-induced miRNA. Mol Cell 14:787–799

Jung HJ, Kang H (2007) Expression and functional analyses of microRNA417 in *Arabidopsis thaliana* under stress conditions. Plant Physiol Biochem 45:805–811

Kapranov P, Cawley SE, Drenkow J, Bekiranov S, Strausberg RL, Fodor SP, Gingeras TR (2002) Large-scale transcriptional activity in chromosomes 21 and 22. Science 296:916–919

Khraiwesh B, Zhu JK, Zhu J (2012) Role of miRNAs and siRNAs in biotic and abiotic stress responses of plants. Biochim Biophys Acta 1819(2):137–148

Kim J, Jung JH, Reyes JL, Kim YS, Kim SY, Chung KS, Kim JA, Lee M, Lee Y, Kim VN, Chua NH, Park CM (2005) microRNA directed cleavage of ATHB15 mRNA regulates vascular development in Arabidopsis inflorescence stems. Plant J 42:84–94

Kong YM, Elling AA, Chen B, Deng XW (2010) Differential expression of microRNAs in maize inbred and hybrid lines during salt and drought stress. Am J Plant Sci 1:69–76

Koyro HW, Ahmad P, Geissler N (2012) Abiotic stress responses in plants: an overview. In: Ahmad P, Prasad MNV (eds) Environmental adaptations and stress tolerance of plants in the era of climate change. doi: 10.1007/978-1-4614-0815-4_1

Kurihara Y, Watanabe Y (2004) Arabidopsis micro-RNA biogenesis through Dicer-like 1 protein functions. Proc Natl Acad Sci USA 101:12753–12758

Lauter N, Kampani A, Carlson S, Goebel M, Moose SP (2005) MicroRNA172 down-regulates glossy15 to promote vegetative phase change in maize. Proc Natl Acad Sci USA 102:9412–9417

Lee RC, Feinbaum RL, Ambros V (1993) The *C. elegans* heterochronic gene lin-4 encodes small RNAs with antisense complementarity to lin-14. Cell 75:843–854

Li H, Deng Y, Wu T, Subramanian S, Yu O (2010) Misexpression of miR482, miR1512, and miR1515 increases Soybean Nodulation. Plant Physiol 153:1759–1770

Li H, Dong Y, Yin H, Wang N, Yang J, Liu X, Wang Y, Wu J, Li X (2011a) Characterization of the stress associated microRNAs in Glycine max by deep sequencing. BMC Plant Biol 11:170

Li T, Li H, Zhang YX, Liu JY (2011b) Identification and analysis of seven H_2O_2-responsive miRNAs and 32 new miRNAs in the seedlings of rice (*Oryza sativa* L. ssp. indica). Nucleic Acids Res 39(7):2821–2833

Liang M, Haroldsen V, Cai X, Wu Y (2006) Expression of a putative laccase gene, ZmLAC1, in maize primary roots under stress. Plant Cell Environ 29:746–753

Lim LP, Glasner ME, Yekta S, Burge CB, Bartel DP (2003) Vertebrate microRNA genes. Science 299(5612):1540

Liu B, Li P, Li X, Liu C, Cao S, Chu C, Cao X (2005) Loss of function of OsDCL1 affects microRNA accumulation and causes developmental defects in rice. Plant Physiol 139:296–305

Liu B, Chen Z, Song X, Liu C, Cui X, Zhao X, Fang J, Xu W, Zhang H, Wang X, Chu C, Deng X, Xue Y, Cao X (2007) *Oryza sativa* Dicer-like4 reveals a key role for small interfering RNA silencing in plant development. Plant Cell 19:2705–2718

Liu HH, Tian X, Li YJ, Wu CA, Zheng CC (2008) Microarray-based analysis of stress-regulated microRNAs in *Arabidopsis thaliana*. RNA 14:836–843

Liu Q, Zhang YC, Wang CY, Luo YC, Huang QJ, Chen SY, Zhou H, Qu LH, Chen YQ (2009) Expression analysis of phytohormone-regulated microRNAs in rice, implying their regulation roles in plant hormone signaling. FEBS Lett 583(4):723–728

Llave C, Xie Z, Kasschau KD, Carrington JC (2002) Cleavage of Scarecrow-like mRNA targets directed by a class of Arabidopsis miRNA. Science 297:2053–2056

Lu C, Tej SS, Luo S, Haudenschild CD, Meyers BC, Green PJ (2005a) Elucidation of the small RNA component of the transcriptome. Science 309:1567–1569

Lu S, Sun YH, Shi R, Clark C, Li L, Chiang VL (2005b) Novel and mechanical stress-responsive microRNAs in *Populus trichocarpa* that are absent from Arabidopsis. Plant Cell 17:2186–2203

Lu SF, Sun YH, Chiang VL (2008) Stress-responsive microRNAs in Populus. Plant J 55:131–151

Lv DK, Bai X, Li Y, Ding XD, Ge Y, Cai H, Ji W, Wu N, Zhu YM (2010) Profiling of cold-stress-responsive miRNAs in rice by microarrays. Gene 459(1–2):39–47

Mallory AC, Vaucheret H (2006) Functions of microRNAs and related small RNAs in plants. Nat Genet 38(Suppl):S31–S36

Mallory AC, Dugas DV, Bartel DP, Bartel B (2004) MicroRNA regulation of NAC-domain targets is required for proper formation and separation of adjacent embryonic, vegetative, and floral organs. Curr Biol 14:1035–1046

Mallory AC, Bartel DP, Bartel B (2005) MicroRNA-directed regulation of Arabidopsis AUXIN RESPONSE FACTOR17 is essential for proper development and modulates expression of early auxin response genes. Plant Cell 17:1360–1375

Margulies M, Egholm M, Altman WE, Attiya S, Bader JS, Bemben LA, Berka J, Braverman MS, Chen YJ, Chen Z (2005) Genome sequencing in microfabricated high-density picolitre reactors. Nature 437:376–380

Martinez J, Tuschl T (2004) RISC is a 5′ phosphomonoester producing RNA endonuclease. Genes Dev 18:975–980

Melda K, Unver T, Budak H (2010) Regulation of barley miRNAs upon dehydration stress correlated with target gene expression. Funct Integr Genomics 10:493–507
Meng Y, Ma X, Chen D, Ping W, Chen M (2010) MicroRNA-mediated signaling involved in plant root development. Biochem Biophys Res Commun 393:345–349
Monteiro CC, Carvalho RF, Gratão PL, Carvalho G, Tezotto T, Medici LO, Peres LEP, Azevedo RA (2011) Biochemical responses of the ethylene-insensitive never ripe tomato mutant subjected to cadmium and sodium stresses. Environ Exp Bot 71:306–320
Motameny S, Wolters S, Nürnberg P, Schumacher B (2010) Next generation sequencing of miRNAs – strategies, resources and methods. Genes 1:70–84
Nam JW, Shin KR, Han J, Lee Y, Kim VN, Zhang BT (2005) Human microRNA prediction through a probabilistic co-learning model of sequence and structure. Nucleic Acids Res 33(11):3570–3581
Naqvi AR, Islam MN, Choudhury NR, Haq QMR (2009) The fascinating world of RNA interference. Int J Biol Sci 5(2):97–117
Navarro L, Dunoyer P, Jay F (2006) A plant miRNA contributes to antibacterial resistance by repressing auxin signaling. Science 312:436–439
Olsen PH, Ambros V (1999) The lin-4 regulatory RNA controls developmental timing in *Caenorhabditis elegans* by blocking LIN-14 protein synthesis after the initiation of translation. Dev Biol 216:671–680
Park W, Li J, Song R, Messing J, Chen X (2002) CARPEL FACTORY, a Dicer homolog, and HEN1, a novel protein, act in microRNA metabolism in *Arabidopsis thaliana*. Curr Biol 12:1484–1495
Park MY, Wu G, Gonzalez-Sulser A, Vaucheret H, Poethig RS (2005) Nuclear processing and export of microRNAs in Arabidopsis. Proc Natl Acad Sci USA 102:3691–3696
Peng T, Lv Q, Zhang J, Li J, Du Y, Zhao Q (2011) Differential expression of the microRNAs in superior and inferior spikelets in rice (*Oryza sativa*). J Exp Bot 62(14):4943–4954
Rajagopalan R, Vaucheret H, Trejo J, Bartel DP (2006) A diverse and evolutionarily fluid set of microRNAs in *Arabidopsis thaliana*. Genes Dev 20:3407–3425
Reinhart BJ, Slack FJ, Basson M, Pasquinelli AE, Bettinger JC (2000) The 21- nucleotide let-7 RNA regulates developmental timing in *Caenorhabditis elegans*. Nature 403:901–906
Reinhart BJ, Weinstein EG, Rhoades MW, Bartel B, Bartel DP (2002) MicroRNAs in plants. Genes Dev 16:1616–1626
Rhoades MW, Reinhart BJ, Lim LP, Burge CB, Bartel B, Bartel DP (2002) Prediction of plant microRNA targets. Cell 110:513–520
Sahi C, Agarwal M, Reddy MK, Sopory SK, Grover A (2003) Isolation and expression analysis of salt stress-associated ESTs from contrasting rice cultivars using a PCR-based subtraction method. Theor Appl Genet 106:620–628
Sahi C, Singh A, Blumwald E, Grover A (2006) Beyond osmolytes and transporters: novel plant salt stress tolerance-related genes from transcriptional profiling data. Physiol Plant 127:1–9
Shiozaki N, Yamada M, Yoshiba Y (2005) Analysis of salt-stressinducible ESTs isolated by PCR-subtraction in salt-tolerant rice. Theor Appl Genet 110:1177–1186
Song L, Han MH, Lesicka J, Fedoroff N (2007) Arabidopsis primary microRNA processing proteins HYL1 and DCL1 define a nuclear body distinct from the Cajal body. Proc Natl Acad Sci USA 104:5437–5442
Stephenson T, McIntyre C, Collet C, Xue GP (2007) Genome-wide identification and expression analysis of the NF-Y family of transcription factors in *Triticum aestivum*. Plant Mol Biol 65:77–92
Sunkar R, Zhu JK (2004) Novel and stress-regulated microRNAs and other small RNAs from Arabidopsis. Plant Cell 16:2001–2019
Sunkar R, Girke T, Jain PK, Zhu JK (2005) Cloning and characterization of microRNAs from rice. Plant Cell 17:1397–1411
Sunkar R, Kapoor A, Zhu JK (2006) Posttranscriptional induction of two Cu/Zn superoxide dismutase genes in Arabidopsis is mediated by downregulation of miR398 and important for oxidative stress tolerance. Plant Cell 18:2051–2065
Sunkar R, Li YF, Jagadeeswaran G (2012) Functions of microRNAs in plant stress responses. Trends Plant Sci 17(4):196–203

Tomari Y, Zamore PD (2005) Perspective: machines for RNAi. Genes Dev 19:517–529
Vaucheret H, Vazquez F, Crete P, Bartel DP (2004) The action of ARGONAUTE1 in the miRNA pathway and its regulation by the miRNA pathway are crucial for plant development. Genes Dev 18:1187–1197
Vazquez F, Gasciolli V, Crete P, Vaucheret H (2004) The nuclear dsRNA binding protein HYL1 is required for microRNA accumulation and plant development, but not posttranscriptional transgene silencing. Curr Biol 14:346–351
Wang X, Zhang J, Li F, Gu J, He T, Zhang X, Li Y (2005) MicroRNA identification based on sequence and structure alignment. Bioinformatics 21(18):3610–3614
Wang L, Liu H, Li D, Chen H (2011) Identification and characterization of maize microRNAs involved in the very early stage of seed germination. BMC Genomics 12:154
Wang F, Liu R, Wu G, Lang C, Chen J, Shi C (2012) Specific downregulation of the bacterial-type PEPC gene by artificial microRNA improves salt tolerance in Arabidopsis. Plant Mol Biol Rep. doi:10.1007/s11105-012-0418-6
Weber MJ (2005) New human and mouse microRNA genes found by homology search. FEBS J 272(1):59–73
Wei JZ, Tirajoh A, Effendy J, Plant AL (2000) Characterization of salt-induced changes in gene expression in tomato (*Lycopersicon esculentum*) roots and the role played by abscisic acid. Plant Sci 159:135–148
Wightman B, Ha I, Ruvkun G (1993) Posttranscriptional regulation of the heterochronic gene lin-14 by lin-4 mediates temporal pattern formation in *C. elegans*. Cell 75:855–862
Xie Z, Kasschau KD, Carrington JC (2003) Negative feedback regulation of Dicer-Like1 in Arabidopsis by microRNA-guided mRNA degradation. Curr Biol 13:784–789
Xie Z, Allen E, Fahlgren N, Calamar A, Givan SA, Carrington JC (2005) Expression of Arabidopsis MIRNA genes. Plant Physiol 138:2145–2154
Xu DQ, Huang J, Guo SQ (2008) Overexpression of a TFIIIA-type zinc finger protein gene ZFP252 enhances drought and salt tolerance in rice (*Oryza sativa* L.). FEBS Lett 582:1037–1043
Xue C, Li F, Tao H, Liu GP, Li Y, Zhang X (2005) Classification of real and pseudo microRNA precursors using local structure sequence features and support vector machine. BMC Bioinformatics 6(1):310
Yamada K, Lim J, Dale JM, Chen H, Shinn P, Palm CJ, Southwick AM, Wu HC, Kim C, Nguyen M (2003) Empirical analysis of transcriptional activity in the Arabidopsis genome. Science 302:842–846
Yan S, Tang Z, Su W, Sun W (2005) Proteomic analysis of salt stress responsive proteins in rice root. Proteomics 5:235–244
Yang Z, Yon WE, Yu B, Chen X (2006) HEN1 recognizes 21–24 nt small RNA duplexes and deposits a methyl group onto the 2′ OH of the 3′ terminal nucleotide. Nucleic Acids Res 34:667–675
Yanga T, Xuea L, An L (2007) Functional diversity of miRNA in plants. Plant Sci 172(3):423–432
Yu B, Bi L, Zheng B, Ji L, Chevalier D, Agarwal M, Ramachandran V, Li W, Lagrange T, John CW, Chen X (2008) The FHA domain proteins DAWDLE in Arabidopsis and SNIP1 in humans act in small RNA biogenesis. Proc Natl Acad Sci USA 105(29):10073–10078
Zhang BH, Pan XP, Wang QL, Cobb GP, Anderson TA (2005) Identification and characterization of new plant microRNAs using EST analysis. Cell Res 15:336–360
Zhang B, Pan X, Wang Q, Cobb GP, Anderson TA (2006) Computational identification of microRNAs and their targets. Comput Biol Chem 30(6):395–407
Zhang Z, Wei L, Zou X, Tao Y, Liu Z, Zheng Y (2008) Submergence-responsive microRNAs are potentially involved in the regulation of morphological and metabolic adaptations in maize root cells. Ann Bot 102(4):509–519
Zhang Z, Yu J, Li D, Zhang Z, Liu F, Zhou X, Wang T, Ling Y, Su Z (2010) PMRD: plant microRNA database. Nucleic Acids Res 38:D806–D813
Zhao B, Liang R, Ge L, Li W, Xiao H, Lin H, Ruan K, Jin Y (2007) Identification of drought-induced microRNAs in rice. Biochem Biophys Res Commun 354:585–590

Zhao B, Ge L, Liang R, Li W, Ruan K, Lin H, Jin Y (2009) Members of miR-169 family are induced by high salinity and transiently inhibit the NF-YA transcription factor. BMC Mol Biol 10:29

Zhou X, Wang G, Zhang W (2007) UV-B responsive microRNA genes in *Arabidopsis thaliana*. Mol Syst Biol 3:103

Zhou L, Liu Y, Liu Z, Kong D, Duan M, Luo L (2010) Genome-wide identification and analysis of drought-responsive microRNAs in *Oryza sativa*. J Exp Bot 61(15):4157–4168

Zhu JK (2001) Plant salt tolerance. Trends Plant Sci 6(2):66–71

Zhu JK (2002) Salt and drought stress signal transduction in plants. Annu Rev Plant Biol 53:247–273

Zhu JK (2007) Plant salt stress. Encyclopedia of life sciences. DOI: 10.1002/9780470015902.a0001300.pub2

Zhu QH, Andrew S, Matthew L, Fan L, Kennedy G, Gubler F, Helliwell C (2008) A diverse set of microRNAs and microRNA-like small RNAs in developing rice grains. Genome Res 18(9):1456–1465

http://www.springer.com/978-1-4614-6107-4

Salt Stress in Plants
Signalling, Omics and Adaptations
Ahmad, P.; Azooz, M.M.; Prasad, M.N.V. (Eds.)
2013, XV, 509 p. 46 illus., 25 illus. in color., Hardcover
ISBN: 978-1-4614-6107-4