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Regulation of Genetic Responses to Salt Stress

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ABSTRACT

Plant response to salinity-induced stress, like most physiological responses, is specie specific. Despite this specificity, the response is often elicitor dependent, which tends to activate a more general response. While stress in plants may be classified as being biotic or abiotic, both types are known to be influenced by signaling pathway. One of the phenotypically well-characterized specific responses in plants is the production of secondary metabolites. However, the overall signaling pattern and its effects on corresponding genes often lead to their differential expression, which turn it specific. This implies that general and specific responses are activated for each situation. Key mediators amongst the chemical entities with specific physiological effects involved in the signaling pathways include jasmonic acid and acetyl salicylic acid, while the more general mediators include plant growth regulators such as auxins and cytokinins. The molecular mechanism of action of these molecules involves promoter activation that bear specific recognition elements, to which transcription factors can bind to enhance or repress the expression of a given gene. The application of high-throughput techniques has shown that microRNA and chromatin remodeling are involved in exposing such regions under different stress conditions. Here, we discuss the observed differences in salt stress tolerance, and sensitivity to high or low exposure to salt in plants, which correlate with varying degrees of the production of secondary metabolites. It is exposed from the perspective of gene expression under plant growth regulators to physiological response. The role of microRNA and chromatin remodeling as signal elements to control gene expression at DNA binding sites, interacting with transcription factors, which may in turn be affected by microRNAs are also discussed.

Keywords: chromatin remodeling, miRNA, salt stress, and secondary metabolism
Abbreviations: **ABA**, abscisic acid; **ABRE**, ABA-responsive element; **AMP**, antimicrobial peptide; **COR**, cold-regulated; **DRE**, dehydration responsive; **MeJA**, methyl jasmonic acid; **ROS**, reactive oxygen species; **RNAi**, RNA interference; **miRNA**, microRNA; **TF**, transcription factor

CONTENTS

INTRODUCTION

Several plant secondary metabolites are a valuable class of highly complex molecules that are generally expensive to produce (Kolewe *et al.* 2008). Developing production of these molecules in plants requires profound knowledge of plant physiology and genetic control. So far, many different chemical compounds with high value for the chemical, pharmaceutical and food industries have been described. Their production is highly influenced by genotype and culture conditions, in a clear interaction of genotype and environment (Baque *et al.* 2010). Combinatorial interactions amongst ion balance, mineral nutrition and growth responses under salt stress are reflected in controlled, conserved and divergent changes of primary and secondary metabolisms during salt stress in plants (Sanchez *et al.* 2008a).

Changes in physiological and genetic expression occur during salt and drought stress. Plant secondary metabolites are produced generally under stress conditions in response to threats posed by predators or microorganisms such as bacteria, fungi or parasites and by abiotic stress such as salinity and drought stress. Although elicitors of these responses may be diverse, the signaling pathways that arise share some features (Santos *et al*. 2009). Some of the key molecules produced by pathway response belong to a class of proteins called AMPs (antimicrobial peptides) that act directly on the plasmatic membrane of microorganisms, affecting gene regulation or biochemical disorders in microorganisms leading than to death. Once signaling pathways have been activated by stress conditions, a cascade of events stimulates a series of genes, which are up-regulated; including oxidative stress enzymes and those involved in the biosynthesis of secondary metabolites, such as flavonoids and essential oils (Chaves *et al*. 2009).

Salinity and drought stresses share similar pathway responses. For example, the development of a protective mechanism during either stress tends to preserve CO_2 accumulation by reducing its diffusion (Chaves *et al.* 2009), resulting in the production of abscisic acid (ABA) in roots.

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Invited Mini-Review

Some species are tolerant to salt stress and produce high amounts of ABA. *Thelungiela* and *Arabidopsis* show differences in salt tolerance, where *Thelungiela* is highly tolerant to salt stress showing reduced jasmonic and ABA production compared to *Arabidopsis* (Arbona *et al.* 2010), furthermore, its gene regulation is mediated by both plant growth regulators (ABA and JA) that may be triggered at key control points (Arbona *et al.* 2010). Time-course evaluation in a cell culture of *A. thaliana* 'T87' line, which demonstrated that the metabolic level altered with time, showing a different metabolic composition depending on the duration, the culture spent under salt stress. This implies that salt stress may enable the different systematic mechanisms in the complex metabolic events induced by salinity to be elucidated, highlighting the potential of time-course metabolic profiling as a powerful tool for analyzing functional genomics and systems biology (Kim *et al.* 2007).

Reactive oxygen species production consequences

Some other alterations may occur, such as cell autophagy, which was observed in *Arabidopsis* as the plant response to saline stress. Autophagy involves protein degradation, probably devoid of reactive oxygen species (ROS) accumulation, which may cause damage to cellular proteins (Liu *et al.* 2009a). These findings showed that culture conditions considerably affect gene regulation and cellular responses during secondary metabolite production. Some metabolites are expressed only at an appropriate time after abiotic or biotic elicitation and are exclusive to that condition (Pedras and Zheng 2010). Intense control of cell death, ROS and secondary metabolism is necessary for bioactive molecules production.

The analysis of metabolome data correlated to shoot biomass in a positive manner to the predictability concentration of Na and K ions, so that highly predictive qualities of models based on metabolome phenotyping may allow the estimation of how much salt stress will be experienced by a plant. In the future, it may thus be possible to use metabolic fingerprinting as a breeding tool to select individual plants that cope best with salt stress (Sanchez *et al.* 2008b). In *Arabidopsis*, glucosinolate was strongly induced by methyl jasmonic acid (MeJA) treatment that triggers the same pathway of salt stress generating indole glucosinolates, which are secondary metabolites induced by plant defense mechanism against threats such as insect attack. In a recent work, proteins involved in stress response were found to be up-regulated (Chen *et al.* 2011). The proteomic approach may be used to obtain insights into physiological processes. The time course of glucosinolate accumulation in *Arabidopsis* leaves allowed the authors to identify 194 different spot of proteins involved in stress response induced by MeJA (Chen *et al.* 2011).

Thellungiella, a known salt-stress-tolerant species, exhibits higher levels of protection and repair mechanisms, including production of ROS scavengers, dehydrins and late embryogenesis abundant proteins, showing marked differences in metabolome including malate, proline, glucose and fructose and mineral PO_4^{3-} and SO_4^{2-}. Although salt stress reduced growth for both *Arabidopsis* and *Thellungiella*, damage in tolerant ones was lower and control of dehydration seems to be the key point for salt tolerance by the accumulation of organic compounds in *Thellungiella* (Lugan *et al.* 2010). Systemic and dynamic responses are caused by various salinity conditions in tobacco plants. It is clear that severity response is completely different from different salt stresses in terms of salt concentrations and durations. An elevation of uracil, uridine and hypoxanthine following seven days of treatment with 500 mM salt probably indicates that such long-term salinity with high salt concentration promotes severe degradation of DNA/RNA (Zhang *et al.* 2011), which are consequence of programmed cell death probably devoid to reactive oxygen species.

Gene regulation and ROS modulation

Interestingly, general responses are induced by exposure of plants to high salinity that may lead to the production of ROS and secondary metabolites including polyphenol compounds with a wide range of physiological properties, such as anti-allergenic, anti-artherogenic, anti-inflammatory, antimicrobial, anti-thrombotic, cardioprotective and vasodilatory effects (Ksouri *et al.* 2007). Some genes are commonly expressed during different plant responses to biotic or abiotic stress. Several responses are consequence of the gene expression and evolutionary adaptation of cells that include general responses shared between different stresses in appropriated strategies upon environmental pressure. *In silico* transcriptome analysis of cDNA library from roots of *Codonopsis lanceolata*, a medicinal plant, showed some genes involved in ROS metabolism or lignin biosynthesis shared responses to stress stimulus, but some others were organ specific (Sathiyamoorthy *et al.* 2011). The saline stresses have a series of consequences that includes reduction of plant growth. In sugarcane the exposure to salinity may be a consequence of metabolic pathway deviation. Sensitive NaCl tolerant clones of sugarcane were exposed to different salt levels and showed effects on the sensitive clone for all parameters analyzed. Total chlorophyll, shoot length and secondary metabolites showed alterations and most of these effects play an important role in scavenging oxidative species (Wahid and Ghazanfar 2006), which is a consequence of saline exposition. In rice, the expression of OsCPK12 seems to modulate salt stress by reducing ROS during salt accumulation, increasing ABA sensitivity, which confers, as a parallel response, plant resistance against blast fungus (Asano *et al.* 2012). In grapevine, high salinity and drought lead to high expression of the mRNA of enzymes involved in ROS scavenging and the transcripts involved in ABA metabolism. Some transcription factors involved in cell development, cell growth and cell wall functions were reported to be differentially expressed under salinity and water deficit stress, altering glucose, malate, and proline levels in water-deficit-treated plants compared to plants under saline treatment (Cramer *et al.* 2007). Analysis of transcriptome of two related *Thellungiela* species, known as salt-tolerant plant, and *Arabidopsis*, a salt-sensitive plant, revealed that ABA biosynthesis, redox control, protein folding, histone genes, and cell-wall synthesis genes are highly expressed in *Thellungiella*, which appears to be superior in managing metabolite composition and maintenance of energy, while *Arabidopsis* expended energy at low stress levels in several pathways (Gong *et al.* 2005). In poplar, an important woody plant, there is evidence that salt-tolerant species have a differential transcriptome and metabolome profile in response to salt and the preparedness involves ABC transporters, metabolite transporters, sugar transporters and transporters with known functions in salt tolerance such as aquaporins, Ca^{2+}, Na^+, and K^+ transporters.

It has been proposed that the downside to a high level of stress preparedness as a result of adaptation to a specific ecological niche is a loss of flexibility and adjustability of the transcriptome to environmental cues (Janz *et al.* 2010). Analysis of polyphenol content and ROS showed that sensitive accession of *Cakile maritima* showed antioxidant (polyphenols) accumulation and antioxidative ability under salinity stress (Ksouri *et al.* 2007). Thus, stimulations of shoot biomass and polyphenol concentration in tissues of 'Jerba' (halophyte) at 100 mM NaCl supports the assumption that stress tolerant plants (such as halophytes) are potentially interesting systems for production of secondary metabolites useful for food and medicinal applications (Ksouri *et al.* 2007). A more global analysis of barley 'Sahara' cultivar, the best adapted cultivar, displayed its tolerance to high internal salt concentrations without apparent cell damage, suggesting that this cultivar may have mechanisms to maintain a higher K^+/Na^+ ratio in the cytoplasm increasing sugars, polyols, and a large number of organic acids, while the sensitive cultivar showed cell damage and

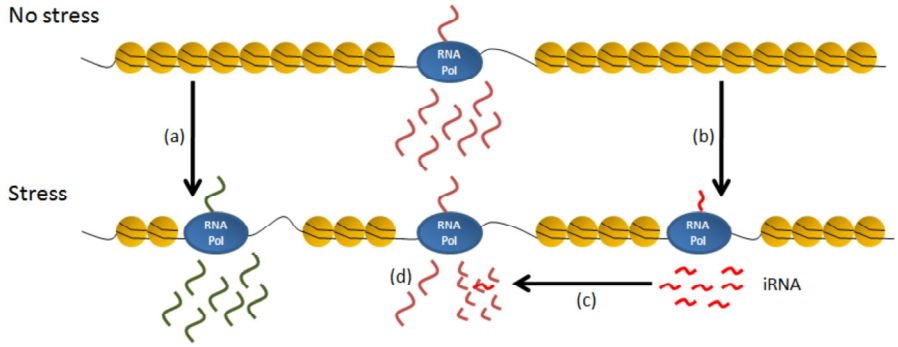

Fig. 1 Genetic changes under abiotic stress. Stress can induce chromatin reorganization associated with induction or repression of gene transcription (a) and changes may be associated with induction or repression of genes for regulatory RNAs (b). miRNAs can also regulate the expression of genes modulating the amount of mRNA available (c) or modifying chromatin (these processes are also associated with the control of gene expression, as previously reported). Stress can also modify the expression of genes through mechanisms of pre-transcriptional control, increasing, decreasing, blocking or inducing the expression of genes (d). All these changes are associated with the plant's response to the stress and metabolites production.

necrosis (Widodo *et al.* 2009).

Increase in salinity considerably affects plant growth and development, inducing secondary metabolism alteration and ROS. At DNA level, some alterations, such as methylation, chromatin remodeling and gene control through RNAi are consequences and causes of pattern of salt response in tolerant genotypes. In *Salvia officinalis* association of NaCl with other salts, mainly $MgCl_2$, increased production of essential oils (EOs) including monoterpenes and diterpenes CA and reduced production of ROS, probably because Mg is a cofactor for terpene synthases and additionally induced plant growth (Tounekti *et al.* 2010).

TRANSCRIPTIONAL AND POST-TRANSCRIPTIONAL CHANGES

Plants interact with the environment by biochemical and genetic modifications. The first of these modifications to be widely studied was transcriptional control, but recently the rapid progress in this area may explain some alterations that could be related to post-transcriptional exchanges during plant production under continuous system (**Fig. 1**). It is important to note that salt stress acclimatization implies, in general, transcriptional regulation under long-term salinity that is mainly dominated by ion accumulation or toxicity, which in turn requires a dose-dependence on salt acclimatization (Sanchez *et al.* 2008c). Microarray analysis of plants with mutations in genes involved in lignin biosynthesis showed that this pathway affects overall distribution of metabolites under saline and drought stress. Additional analysis of double mutants for *f5h* (ferulate 5-hydroxylase) and *sct* (sinapoylglucose choline sinapoyltransferase) have shown plants with secondary metabolism being affected only under specific conditions such as salt stress and drought (Huang *et al.* 2009). Global transcriptome analysis under $NaHCO_3$ stress in wild soybean showed that signal transduction genes, transcription genes and secondary metabolism genes are among the up-regulated class of genes. Transcription factors (TFs) from 29 different families were found to be induced at the first stages of stress such including AP2-EREBP, WRKY, bZIP, MYB and MYB related. Some knowm miRNA including miR166a, miR168 and miR2108 were also induced by $NaHCO_3$ stress (Ge *et al.* 2010). Full-length cDNA through FOX hunting system was able to identify salt tolerant genes in *Thellungiella* by gain-of-function through plant transformation (Du *et al.* 2008). Transcription factors are expected to be involved in stress responses, and few MYB, a salt stress TF gene family generally associated to salt tolerance, have been functionally characterized. Lippold *et al.* (2009) observed that there was no increase in the sensitivity of growth to salt and drought stresses of AtMyb41-over-expressing plants compared with wild-type controls. This is perhaps not surprising given the multigenic nature of abiotic stress tolerance. Large-scale transcriptomic analyses of *Medicago* roots subjected to salt stress enabled the identification of several TFs under analysis of salt-stress responses in root apexes versus whole roots, linked to the spatial regulation of gene expression after salt stress in *Medicago truncatula* (Gruber *et al.* 2009). Several transcripts were identified from *L. maritima* that are novel in context of salt and drought-response regulation, including signal transduction elements. However, a bZIP transcription factor that was up-regulated by an increase in salt probably regulates negatively the genes involved in ion homeostasis (Popova *et al.* 2008). A transcription factor identified in tomato is sensitive to ABA and is involved in both biotic and abiotic stresses, which clearly demonstrated that a pleiotropic effect could be involved in plant stress response (AbuQamar *et al.* 2009).

SMALL RNAs AND ITS IMPLICATION IN SALT STRESS RESPONSE

The importance of post-transcriptional gene regulation in response to environmental stress has been highlighted with the discovery of small RNAs, microRNAs (miRNAs) and small interfering RNAs (siRNAs), which consist of 21 to 24-nucleotide non-coding RNAs that are responsible for the phenomenon known as RNA interference (RNAi); this functions by causing either transcriptional or post-transcriptional gene silencing (Baulcombe 2004). Recently, the role of miRNAs in the regulation of genes involved in plant stress responses and how the stress affects small RNAs has become the subject of intense investigation. Readers are referred to recent reviews, which deal with this specific subject (Shukla *et al.* 2008; Covarrubias and Reyes 2010; Zhu *et al.* 2011; Khraiwesh *et al.* 2012).

Stress identification and target prediction of miRNAs in poplar indicated crosstalk between biotic and abiotic stress (Lu *et al.* 2008; Sanan-Mishra *et al.* 2009). RNA interference mediated post-transcriptional control is mediated in part by Dicer, Argonaute and RNA-dependent RNA polymerases, which together constitute an important regulatory process that checks transcript accumulation in cells (Kapoor *et al.* 2008). Microarray analysis in rice showed that most of the studied genes involved in RNA silencing do not show expression level during salt stress except *OsAgo2* (argonaute family). *Cis* natural antisense transcripts (*cis*-NAT genes) are a class of transcripts that are metabolized by RNAi complex. Their occurrence in the genome is estimated at about 7 to 30%. Most *cis*-NAT transcripts (about 45%) were identified to act under these treatments, and results found by these authors support the hypothesis that these elements are part of an important mechanism in gene regulation (Jin *et al.* 2008). In tobacco, some miRNAs are up-regulated under salinity and drought stresses, where miR395 was found to be highly sensitive to salt treatment, being up-regulated nearly 100 times more than other

miRNAs and inhibited under low salinity concentrations (Frazier *et al.* 2011). Global miRNA analysis in rice revealed that two of the total miRNAs are unregulated by drought, six up-regulated by cold. While miR408 was not regulated by salt and drought conditions, miR395 was up-regulated under salinity and cold stress. On the other hand, miR171 was only up-regulated under salinity (Shen *et al.* 2010). In transgenic *Arabidopsis* the over-expression of two miR395 members reduced sulfate transport and assimilation, retarded germination of seedling development under salinity stress. This finding suggests that proteins involved in sulfate assimilation are the target for this miRNA, and that miR395c seems to affect ABA (Kim *et al.* 2010a), while miR402 is probably the target of DEMETER genes, which are required for proper methylation distribution in genome.

Mutants of *dlm3* and transgenic plants over-expressing miR402 showed high resistance to salt stress (Kim *et al.* 2010b). Although miR395 was not affected by salt and ABA stress in a reverse way to APS1 (ATP sulphorylase) involved in sulfate metabolism in poplar, miR398 was up-regulated in the short term by salt stress and ABA treatment, showing a completely opposite response in *Arabidopsis* (Jia *et al.* 2009). Moreover, Jagadeeswaran *et al.* (2008) demonstrated that miR398 was also involved in bacterial response, probably by up-regulation of target genes.

In maize, the *rolled leaf 1* (*rld1*) transcription factor was enhanced after salt induction, and miR166 was down-regulated under this condition (Ding *et al.* 2009). It is consistent with the fact that, miR166 cleaves rld1 mRNA (Juarez *et al.* 2004). The MiR169 family contains a series of miRNA elements that may be involved in salinity stress response. They contain an ABA responsive element (ABRE), dehydration responsive *cis* acting elements (DRE) and NF-Y, CCAAT box salt responsive genes, which are cleaved specifically under this condition (Zhao *et al.* 2009). The osa-MIR396 that has an ABRE element was found to be down-regulated under salt stress, inducing genes that confer tolerance to this condition (Gao *et al.* 2010), like osa-MIR393 (Gao *et al.* 2011). Finally, the conservation of miRNA amongst different groups indicates that these regulatory elements must have played an important role during the evolution of plant development and responses to environmental stress, especially in legumes (Arenas-Huertero *et al.* 2009).

Several differentially regulated miRNAs have been identified in salt-stressed plants (see miRBase at http://www.mirbase.org/). *In silico* studies indicated for the first time that siRNAs could play a role in abiotic stress responses. In these studies miRNAs were associated with their target genes expressed under stressed and unstressed conditions (Jones-Rhoades and Bartel 2004; Sunkar and Zhu 2004). Environmental stresses in plants provoked over- or under-expression of certain miRNAs or synthesis of new miRNAs to cope with stress. However, this does not necessarily mean miRNA is involved in stress adaptation responses.

The transcriptional down-regulation of miR398 by oxidative stresses in *Arabidopsis* was one of the first reports linking miRNAs to stress tolerance. miR398 was found to target two closely related Cu/Zn-superoxide dismutase coding genes, cytosolic CSD1 and chloroplast CSD2 (which can detoxify superoxide radicals), and a reduced level of miR398 generated improved oxidative stress tolerance in transgenic lines when compared with the wild-type plants (Sunkar *et al.* 2006). In rice, miR169g was confirmed as the only member of the miR169 family induced by drought (Zhao *et al.* 2007). miR398 was found to be differentially expressed in leaves (up-regulated) and roots (down-regulated) (De Paola *et al.* 2012) In addition, 21 miRNAs belonging to 11 miRNA families in *Arabidopsis* were predicted to be up-regulated under UV-B stress conditions (Zhou *et al.* 2007). Recently, Jagadeeswaran *et al.* (2009) reported that miR398 levels were down-regulated in response to ozone fumigation. It should be treatment and salt stress as well as in bacterial infiltrations. Additionally, increased CSD1, but not CSD2- mRNA levels negatively correlated with decreased miR398 levels under ozone, salinity and biotic stress conditions. In contrast, the down-regulation of miR398 under salt stress was absent in *Populus tremula*, in which miR398 was first induced during 3–4 h of salt stress, then declined after 48 h and finally accumulated again during prolonged stress (72 h) (Jia *et al.* 2009). This suggested specific regulation of miRNA expression in different plant species, especially comparing *Arabidopsis* (an annual herbaceous plant) with *P. tremula* (a perennial woody plant) (Jia *et al.* 2009).

Sunkar and Zhu (2004) generated a library of small RNAs from *Arabidopsis* seedlings exposed to salt stress and identified several miRNAs that are responsive to abiotic stress. Among the miRNAs, they found that miR393 was up-regulated by salinity treatments while miR389a was down-regulated. Similarly, several of the miRNAs discovered in this study were either up-regulated or down-regulated by abiotic stresses, suggesting that they may be involved in stress-responsive gene expression and stress adaptation. Consistent with this notion, the Arabidopsis *hen1-1* and *dcl1-9* mutants that are impaired in the production of some miRNAs are hypersensitive to abiotic stresses (Sunkar and Zhu 2004). In maize, an analysis of the miRNA profile in salt-tolerant and salt-sensitive lines indicated that members of the miR156, miR164, miR167, and miR396 families were down-regulated, while miR162, miR168, miR395, and miR474 families were up-regulated in salt-treated maize roots (Ding *et al.* 2009). Stress up-regulated miRNAs are expected to target negative regulators of stress responses or positive regulators of processes that are inhibited by stresses. On the other hand, stress down-regulated miRNAs may repress the expression of positive regulators and/or stress up-regulated genes.

Analyzing the effects of 117 miRNAs under stress conditions with miRNA chips, Liu *et al.* (2008) detected 10 high-salinity regulated miRNA (miR156, miR159, miR165, miR167, miR168, miR171, miR319, miR393, miR394 and miR396). miR168, miR171, and miR396 responded to all of the stresses. Expression profiling by RT-PCR analysis showed a cross talk among the high-salinity, drought, and cold stress signaling pathways. The analysis also revealed temporal expression patterns of these miRNAs. The levels of miR156h, miR167a, miR167c, miR167d, miR168 and miR171b gradually increased from 2 to 24 h after exposure to high-salinity treatment, while expression of miR396a peaked at 24 h. miR167a levels accumulated after 2 h of drought stress and were greatly increased after 24 h of treatment, while miR168 first increased then returned to a normal level after 6 h (Liu *et al.* 2008).

In *Phaseolus vulgaris*, Arenas-Huertero *et al.* (2009) reported an increased accumulation of miRS1 and miR159.2 in response to salt treatment of 4-day-old seedlings exposed to 200 mM NaCl. They also detected a salt stress-induced expression of miR393, a miRNA previously shown to respond to a variety of stress conditions in *Arabidopsis* (Sunkar and Zhu 2004). It is interesting that miR159.2 is present in the miR159a precursor in *P. vulgaris*, *Glycine max* and other leguminous species. In *Populus trichocarpa* plantlets exposed to salt stress treatment (300 mM NaCl for 14 h), miR530a, miR1445, miR1446a-e, miR1447, and miR171l-n were down-regulated, whereas miR482.2 and miR1450 were up-regulated (Lu *et al.* 2008). Additionally, it was observed that some of the stress-responsive miRNA families are deeply conserved among various plant species, such as *Arabidopsis*, rice, and *Populus*. Others are specific to *Populus* or particularly trees, possibly as the result of adaptation to long-term growth and survival in stressful environments.

In switchgrass, a NAC transcription factor domain was identified as a target for miR164 (Matts *et al.* 2010). NAC type proteins have also been recognized as playing a key role in abiotic stress tolerance including drought and salinity. Thus, regulation of NAC by miRNA-mediated cleavage of mRNAs together with data showing differential regulation of NAC factors in response to drought and salt stress indicate that it might participate in the regulation of envi-

ronmental adaptation through miRNA pathways (Golldack *et al*. 2011).

The expression of the two members of the miR393 family found in rice (osa-MIR393 and osa-MIR393b) was analyzed during salinity stress (Gao *et al.* 2011). miR393 is a conservative miRNA family that occurs in a variety of different plants. The osa-MIR393 expression level changed under salinity stress, whereas the levels of osa-MIR393b were not found to be changed. Target genes of osa-MIR393 were predicted, and some of these putative targets are abiotic related genes, encoding transport inhibitor response proteins, oxido-reductase, the phytosulfokine receptor precursor and GRF-interacting factor (GIF). In addition, transgenic rice and *Arabidopsis thaliana* were generated to overexpress osa-MIR393 and presented a more salt-sensitive phenotype (Gao *et al.* 2010, 2011). In salt-tolerant cotton cultivar 17, miRNA was identified and the authors observed that down-regulation of miRNAs under salt stress caused accumulation of target mRNAs, contributing to cotton's adaptation under salt stress affecting transcription factors and enzymes involved in plant development (Yin *et al.* 2012).

In order to establish a complete scenario on how siRNAs act to regulate abiotic stress responses in plants, the identification of target genes is crucial. In addition, this identification should also be associated with solid biochemical and genetic data, which will allow a thorough understanding of the transcriptional, post-transcriptional and post-translational changes during stress conditions. The identification of a set of stress-responsive miRNAs could provide the first line of information necessary for the development of stress-tolerant plants using molecular breeding approaches.

CHROMATIN MODIFICATIONS AND RESPONSES TO ABIOTIC STRESS

Abiotic stresses, such as drought, cold and high salinity, affect plant growth and induce various biochemical and physiological responses in plants, including changes in gene expression mediated by transcriptional and post-transcriptional regulation. Recent studies have demonstrated the coordination of gene expression and chromatin regulation in response to abiotic stresses (Chen *et al*. 2010; Pavangadkar *et al.* 2010; Kaldis *et al.* 2011). Higher-order chromatin folding prevents access of transcription machinery to DNA. Two categories of proteins may act by modifying this state: a) Chromatin remodeling complexes that destabilize chromatin by displacement or removal of histones; b) Enzymes that covalently modify histones by acetylation, methylation, phosphorylation, ubiquitinylation, and other post-translational modifications (Pavangadkar *et al.* 2010). Hyperacetylation of histones H2B, H3, and H4 has been generally associated with transcriptionally active chromatin (Struhl 1998; Zhu *et al.* 2008), whereas the chromatin of inactive regions is enriched in deacetylated histones (Strahl and Allis 2000; Zhu *et al.* 2008). Additional support for the existence of a direct molecular link between histone acetylation status and transcription regulation (Glass and Rosenfeld 2000; Courey and Jia 2001; Jepsen and Rosenfeld 2002; Zhu *et al.* 2008) is provided by the finding that many transcriptional co-activators, including GCN5, PCAF, CBP/p300, and SRC-1/ACTR, possess intrinsic histone acetyl transferase activity. Moreover, transcriptional repressors such as NuRD, SIN3, Groucho/Tup1, and SMRT/N-CoR associate with histone de-acetylases (Glass and Rosenfeld 2000; Courey and Jia 2001; Jepsen and Rosenfeld 2002; Zhu *et al.* 2008).

In *Arabidopsis*, the gene SKB1 acts by altering the methylation status of H4R3sme2 and LSM4 and pre-mRNA splicing due to salinity stress conferring salt tolerance (Zhang *et al.* 2011). In the same species, NAP 1 (Nucleosome Assembly Protein 1) is involved in plant response to abscisic acid (ABA), a phytohormone important in stress adaptation (Liu *et al.* 2009b). ABA and salt stress response also correlate with the action of HDAs (histone deacetylases) in *Arabidopsis* (Chen *et al.* 2010). The actions of these enzymes normally result in gene repression by deacetylation of nucleosome core histones (Chen and Tian 2007; Chen *et al.* 2010). Related to drought responses, Lysine modifications on the Histone H3 N-Tail for the genes *RD29A*, *RD29B*, *RD20* and *RAP2.4* (*Arabidopsis* drought stress-inducible genes) are reported where the nucleosome positioning changes have also been reported (Kim *et al.* 2008).

Zhu *et al.* (2008) reported that HOS15, a WD40-repeat protein, functions to control gene expression through histone deacetylation in chromatin. The *hos15* mutant plants accumulate higher levels of transcripts of many stress-regulated genes and are hypersensitive to freezing temperature. Pavangadkar *et al.* (2010) demonstrated that overexpression of CBF1 transcription factor resulted in a constitutive increase in H3 acetylation and decrease in nucleosome occupancy, consistent with the constitutive activation of COR (cold-regulated gene promoter) gene expression. In contrast, in soybean a protein GmPHD5 (*Glycine max* plant homeodomain 5) is able to recognize specifically a methylated histone H3K4 and recruit chromatin remodeling factors and transcription factors to regulate genes under salt stress by chromatin remodeling between histone methylation and histone acetylation (Wu *et al.* 2011).

In conclusion, plant metabolic production under salt, biotic or other abiotic stress is a multivariate phenotype and its response is a fine evolutionary condition that induces biochemical alteration including scavengers such as ROS, which induce cell autophagy, chromatin remodeling and miRNA alteration. This in turn leads to differential gene expression, which may affect the production of metabolites, involving all aspects of genotype and growth conditions for higher plant production.

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