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Epigenetic Chromatin Regulators as Mediators of Abiotic Stress Responses in Cereals

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1. Introduction

Plants are constantly exposed to environmental changes and have to adapt to a multitude of abiotic and biotic stresses. Due to their sessile nature plants had to develop sophisticated ways to respond and adapt to a variety of external stress factors that would otherwise compromise proper development, reproductive success and ultimately survival.
Years of rigorous research have demonstrated that abiotic stress such as drought, high salinity, temperature extremes, UV irradiation and oxidative stress, affect various cellular processes in plants and induce alterations in gene expression programmes in order to activate the plants defense mechanisms to survival. Extensive studies based on forward genetic, reverse genetics, and biochemical investigations of individual loci as well as genome-wide approaches, especially in the model-plant Arabidopsis, have revealed a plethora of genes that are involved in abiotic stress response pathways and acquisition of stress tolerance. These include a wide range of stress-responsive genes encoding transcription factors and functional proteins whose transcription is altered during abiotic stress [1].
Growing evidence from recent studies has indicated that regulation of expression of stress-responsive genes is often accomplished by epigenetic mechanisms which modulate chromatin structure or regulate the level of mRNA accumulation at the postranscriptional level [2, 3, 4].
In eukaryotes nuclear DNA is organized in chromatin, a tightly packed higher order structure which permits genomic DNA to fit within the nucleus. The fundamental unit of chromatin is the nucleosome which is composed of 147 base pairs of DNA that is wrapped almost twice around an octamer of histone proteins. The octamer consists of two copies of each of histone H2A, H2B, H3 and H4. Chromatin higher-order structure switches between condensed and relaxed states and plays a crucial role in the epigenetic regulation of gene expression [5Kouzarides 2007]. Alterations in chromatin structure affect the accessibility of the transcriptional machinery (transcription factors, RNA polymerase) to nucleosomal DNA and determine the levels of gene expression in response to developmental and environmental stimuli.
Chromatin modulation is achieved by a variety of mechanisms including: DNA methylation catalyzed by DNA cytosine methyltransferases, histone post-translational modifications catalyzed by a wide range of enzymes specific for each modification, alterations in histone-DNA interactions which facilitate nucleosome sliding and are catalyzed by chromatin

remodeling complexes, histone variants, and small RNA related pathways (siRNAs and miRNAs) which act directly on chromatin and induce RNA-dependent DNA methylation (RdDM) [[5]Kouzarides et al, 2007; [6]Pfluger and Wagner, 2007; [7]Law and Jacobsen, 2010; [8]Chapman and Carrington, 2007; [9]Henderson and Jacobsen, 2007; [10]Kasschau et al., 2007; [11]Chinnusamy and Zhu; 2009]. In addition, small RNAs also regulate gene expression at the posttranscriptional level through mRNA degradation and/or translational inhibition [[12]Voinnet 2009; [13]Bartel 2009].

Research on the epigenetic regulation during plant development and in response to abiotic stress has focused on exploration of chromatin modulation at specific loci and the characterization of chromatin modifiers during development and under stress conditions[[2; 3]]. In recent years the advancement of –omics technologies [transcriptomics-microarrays/whole-genome tilling arrays, next generation sequencing (NGS), chromatin immunoprecipitation (ChIP) assays combined with sequencing technology (ChIP-seq), and bioinformatics tools] contributed greatly to these efforts and led to the transition from epigenetics (study of individual locus /small-scale) to epigenomics (study of whole epigenomes/global-scale) [reviewed in [14]Tsaftaris et al., in press]. Large-scale epigenomics studies have established the genome-wide profile of DNA methylations, histone modifications and small RNA patterns, in different developmental stages or under abiotic stress conditions, primarily in the model-plant Arabidopsis [[15]Cokus et al. 2008; [16]Lister et al., 2008; [17]Zhang et al., 2007; [18]Bernatavichute et al., 2008; [19]Zhang et al.,2009; [20]Yang et al., 2010; [21]Van Dijk et al., 2010; [22]Roudier et al., 2011] but also in the cereal model-plant Brachypodium [[23]Zhang et al., 2009b] and in agronomically important cereal crops like rice [[24]Li et al., 2008, [25]Sunkar et al., 2008; [26]He et al., 2010] maize [[27]Wang et al.,2009; [28]Wang et al., 2011] wheat [[29]Yao et al., 2010] and barley [[30]Schreiber et al., 2011]. Together, epigenetics and epigenomics studies have provided a wealth of information about epigenetic regulation in response to developmental and environmental stimuli, mostly in Arabidopsis. Recently, the availability of the rice and maize genomes and epigenomes provided the opportunity for exploring this exciting area in monocots as well, and data on epigenetic regulation in response to abiotic stress in cereals have started to come into sight.

In this review we summarize the current progress on epigenetic regulation in response to abiotic stresses such as drought, cold, and high salinity, in Arabidopsis, and present the emerging information on the epigenetic regulatory mechanisms induced upon abiotic stress in cereals such as rice, maize, wheat and barley. Expanding our understanding of the epigenetic regulation associated with abiotic stress responses in cereals of agronomic importance could have a significant impact in breeding for improved varieties with increased stress tolerance. In view of the global climate change where abiotic stresses are expected to increase dramatically, this undertaking would be of paramount importance.

2. Histone modifications in response to abiotic stress

2.1 Gene activation and deactivation marks

Histone post-translational modifications usually take place on histone tails protruding from nucleosomes, and include methylation, acetylation, phosphorylation, ubiquitination, biotinylation, and sumoylation on specific lysine, arginine, serine and threonine residues [[31]Zhang et al., 2007a; [32]Berger et al., 2007]. A complex pattern of site-specific combinations of histone modifications on different residues known as the 'epigenetic histone code' leads to specific chromatin states in response to intrinsic (developmental) and external

(environmental signals) which regulate transcriptional activity and are inherited by daughter cells [33Strahl and Allis 2000].

The best characterized histone modifications associated with the response of plants to abiotic stress are the histone acetylation/deacetylation and histone methylation/demethylation reversible modulations at individual loci [2, 3Chinnusamy et al. 2008; Chinnusamy and Zhu 2009]. Histone acetylation carried out by histone acetyltransferases (HATs) is associated with gene activation, whereas histone deacetylation, performed by histone deacetylases (HDACs) is associated with gene silencing [34Chen and Tien, 2007]. Histone methylation/demethylation is catalyzed by specific histone methyltransferases (HMTs) and histone demethylases (HDMs), respectively. Tri-methylation of H3 at lysine 4 (H3K4me3) which is catalyzed by a specific histone methyltransferase of the Trithorax (TrxG) group leads to gene transcription, whereas trimethylation of H3 at lysine 27(H3K27me3) by a specific methyltransferase of the Polycomb group (PcG), which antagonizes TrxG, leads to gene repression [35Avramova 2009; 36Alvarez et al., 2010; 37Pontvianne et al., 2009; 38Liu et al., 2010; 39Kapazoglou et al., in press].

Abiotic stress such as drought, cold, heat, high salinity, oxidative stress and UV irradiation, alter the histone acetylation and/or methylation pattern within the promoters or coding regions of genes, thereby causing gene activation or gene silencing. In addition, abiotic (and biotic) stress factors trigger the production of certain phytohormones such as abscisic acid (ABA), jasmonic acid (JA), salicylic acid (SA), gibberellic acid (GA) and ethylene, which mediate the regulation of gene expression during the adaptive responses of plants to various abiotic stresses. It has been proposed that histone acetylation/deacetylation through the action of HATs and HDACs, and histone methylation/demethylation through the action of HMTs and HDMs, respectively, epigenetically regulates the responses to various stresses as well as the integration of hormonal signals controlling stress-responsive genes [2Chinnusamy et al. 2008; 3Chinnusamy and Zhu 2009; 11Chinnusamy and Zhu 2009].

Much research has been conducted in Arabidopsis on the effects of abiotic stress on histone modifications at specific chromatin loci. For example, ChIP assays detected histone modifications on the N- terminal tails of H3 in four drought–stress responsive genes, namely, *RESPONSIVE TO DEHYDRATION(RD)29A, RD29B, RD20* and *AP2 DOMAIN-CONTAINING TRANSCRIPTION FACTOR*, *Atg20880*. In particular, the histone activation marks H3K23ac and H3K27ac were enriched in the coding regions of *RD29B, RD20* and *Atg20880* in response to drought stress and these changes were associated with increased expression of these genes under dehydration conditions [40Kim et al., 2008]. Enrichment for H3K4me3 was also observed at *RD29A* and *Atg20880* chromatin and it occurred after full activation of these genes under conditions of drought. In another study, histone modifications were detected in two cold-responsive genes *COLD-REGULATED (COR)15A* and *ATGOLS3* (encoding galactinol synthase) during exposure to low temperature conditions [41Taji et al., 2002]. H3K27me3, a gene silencing mark, was found to be decreased on the chromatin of both genes and this reduction was associated with reduced expression under cold stress. Another report revealed that phosphorylation of histone H3 at serine 10, phosphoacetylation of H3 at serine 10 and lysine 14, and acetylation of histone H4 were enriched as a response to cold, high salinity, and exogenous ABA application, in Arabidopsis and tobacco cells. The induction of these histone modifications correlated with up-regulation of stress-responsive genes [42Sokol et al., 2007].

Histone modification alterations were also reported in cereals exposed to abiotic stress. Submergence of rice seedlings induced H3K4me3 and H3 acetylation in the 5′ and 3′ regulatory regions and coding regions of the *ALCOHOL DEHYDROGENASE 1* (*ADH1*) and

PYRUVATE DECARBOXYLASE (PDC1) genes. These modifications correlated with upregulation of *ADH1* and *PDC1* and were restored to pre-stress levels after seedlings were reinstating to areation, underlying the dynamic nature of histone methylation and acetylation modifications [43Tsuji et al., 2006]. In maize, exposure to UV irradiation resulted in increased H3 and H4 acetylation within the promoter and coding regions of UV-B-induced genes in a maize-UV-B-tolerant line, whereas such enrichment was not detected in a UV-B-sensitive maize line [44Casati et al., 2008].

Finally, genome-wide analysis using ChIP and deep sequencing (ChIP-Seq) unraveled the global epigenomic map of H3Kme1, H3K4me2 and H3K4me3 during drought stress and non-stress conditions, in Arabidopsis. The H3K4me1 and H3K4me2 were found to be more widely distributed than the H3K4me3 mark. Upon dehydration stress a substantial change in H3K4me3 abundance was observed, whereas there were only moderate changes in H3K4me1 and H3K4me2 levels. In addition, whereas for most transcribed genes the H3K4me3 mark was more prominent at the 5'-ends, for drought- and ABA-induced genes H3K4me3 had an atypically broader distribution profile [21van Dijk et al., 2010].

2.2 Histone modification enzymes

Histone acetyltransferases (HATs)

Histone acetyltransferases (HATs) transfer an acetyl moiety to the ε-amino group of highly conserved lysines in the N-terminal extensions of nucleosomal core histones, thereby neutralizing the positive charge of lysines and resulting in less affinity to the negatively charged DNA molecules. This results in relaxation of chromatin structure and subsequent transcriptional activation. HATs comprise a superfamily including the GNAT/MYST, CBP and TFII250 families and are often subunits of large protein complexes.

AtGCN5, a member of the GNAT/MYST subfamily, is the best studied HAT protein in Arabidopsis and plays a role in gene activation in response to environmental changes such as cold [45Vlachonasios et al., 2003]. AtGCN5 associates *in vitro* with the transcriptional co-activator proteins ADA2a and ADA2b. *ada2b* mutants were found to exhibit hypersensitivity to salt and abscisic acid and had altered responses to low temperature stress [46Hark et al., 2009]. Elongator, another histone acetyltransferase complex consisting of six subunits and highly conserved in eukaryotic organisms, was implicated in abiotic stress response. Mutations in the core subcomplex ABO1/ELP1 and ELP2, but not in the accessory subcomplex ELP4 and ELP6, increased ABA-induced stomatal closure. These mutants also displayed increased tolerance to oxidative stress [47Zhou et al., 2009]. A recent report showed that *ADA2b* positively regulates salt-induced gene expression by maintaining the locus-specific acetylation of histones H4 and H3b. ChIP assays demonstrated that the promoter and coding regions of *COR6.6 (COLD RESPONSIVE 6.6)*, *RAB18 (RESPONSIVE TO ABA 18),* and *RD29b* genes had reduced levels of histone H3 and H4 acetylation in *ada2b-1* mutants relative to wild-type plants [48Kaldis et al., 2011].

Our group has identified *HAT* gene homologues from barley. Representative members of the GNAT/MYST family, namely *HvMYST*, *HvELP3* and *HvGCN5*, were isolated and gene expression was examined in different stages of seed development and in response to ABA treatment. Exposure of barley seedlings to exogenus ABA resulted in marked induction of all three *HAT* genes. *HvELP3* was the one mostly affected by the application of the hormone and had expression levels four times as much in the ABA-treated tissue than the untreated controls. *HvGCN5* and *HvMYST* were also up-regulated by approximately two-fold. These

data implied possible ABA-dependent regulation of barley histone acetyltransferases during seed development and abiotic stress response [49Papaefthimiou et al., 2010].

Histone deacetylases (HDACs).

Histone deacetylases (HDACs) reverse the effect of HATs by removing the acetyl group on histones resulting in condensed chromatin structure and gene silencing [34Chen and Tian, 2007]. Eukaryotic HDACs can be grouped into three major families based on their primary homology to the yeast HDACs: 1) the RPD3/HDA1 family, 2) the SIR2 family and 3) the plant specific family HD2 (50Pandey et al., 2002).

Sequence and phylogenetic analysis of the rice genome identified the respective three HDAC families in rice [51Fu et al., 2007]. HDA1 is further subdivided in four classes Class I, Class II and Class III , and ClassIV, and HD2 in two classes HD2a and HD2b. In maize, 15 HDAC genes have been identified (10 HDA1, 1 SIR2, and 4 HD2-like and a number of HDA1 members have been biochemically characterized [52Lusser et al., 2001; 53Rossi et al., 2003; 54Varotto et al., 2003].

Functional analysis using silencing or overexpression transgenic lines in Arabidopsis has demonstrated that both *HDA1* and *HD2* genes are associated with the response to abiotic (as well as biotic stress). For example, *AtHDAC19* was proposed to mediate jasmonic acid (JA) and ethylene signaling during pathogen defense (55Tian et al. 2005; 56Zhou et al. 2005). Overexpression of *AtHDA19* resulted in reduced histone acetylation levels and upregulation of the stress-related genes *ERF1* (*Ethylene Response Factor-1*) and PR (*Pathogenesis Related*). Conversely, silencing of *AtHDA19* led to increased histone acetylation and downregulation of *ERF1* and *PR*. *AtHDA6*, another HDA1-Class I, was shown to be required for jasmonate response, senescence, and flowering. *AtHDA6* was induced by exogenous JA and ethylene [57Wu et al. 2008]. In addition, in *hda6* mutants and in HDA6-RNAi plants the Arabidopsis JA-responsive genes *PDF1.2*, *VSP2*, *JIN1*, and *ERF1* were downregulated, suggesting an indirect involvement of HDAC6 in JA-responsive gene regulation.

Histone modification changes that take place as a response to abiotic stresses are often found to be induced by phytohormones, such as ABA [2Chinnusamy et al., 2008]. ABA affects a wide range of processes in plants like germination, vegetative to reproductive transitions, seed development, seed dormancy and abiotic stress tolerance. For example, *AtHD2C*, belonging to the HD2 family was proposed to play a role in ABA signaling and abiotic stress, in Arabidopsis (58Sridha and Wu 2006). ABA treatment caused severe reduction in expression of *AtHD2C*, whereas overexpression of *AtHD2C* resulted in enhanced abiotic stress tolerance to salt and drought stress, as well as repression of several ABA-responsive genes and induction of others (Sridha and Wu 2006). *AtHOS15* encoding a protein similar to human transducing-β-like protein (TBC), a component of a repressor protein complex involved in histone deacetylation, was reported to mediate ABA-dependent deacetylation in response to cold stress [59Zhu et al., 2008]. The expression of *AtHOS15* is increased by cold, high salinity, and ABA treatment and *hos15* mutants are hypersensitive to freezing stress. In addition *hos15* mutants displayed increased H4 acetylation levels and concurrent increase of *RD29A* expression levels, suggesting a role for *HOS15* in regulating chromatin acetylation levels and gene expression under abiotic stress.

Furthermore, in a recent report, *AtHDA6* was shown to be involved in modulating the levels of H3K9, 14 ac and H3K4me3 (gene activating marks) and of H3K9me2 (histone deactivation mark) in response to ABA and salt-stress [60Chen et al., 2010]. The *hdac6* mutant and RNAi HDAC6 lines were hypersensitive to ABA and salt stress, and the expression of ABA- and

abiotic stress-inducible genes, *ABI1, ABI2, KAT1, KAT2, DREB2A, RD29A, RD29B* was decreased when these plants were subjected to ABA or salt stress as compared to wild-type plants. Moreover, both ABA application and salt stress increased the gene activation marks, H3K9,14 ac and H3K4me3, in the promoter and coding regions of some of the stress-inducible genes mentioned above. However, such increase was not observed in the *hdac6* mutant lines. Together these observations indicate that *HDAC6* is required for ABA and stress-induced histone acetylation, and most likely functions indirectly by suppressing a repressor of histone acetylation. Ultimately, this leads to gene activation of stress-responsive genes and stress tolerance [60Chen et al., 2010].

Studies on *HDAC* genes in relation to stress and stress-related hormones have been recently reported in cereals as well. Expression analyses of 18 rice *HDAC* genes from HDA1, SIR2 and HD2 families demonstrated distinct spatial expression patterns and differential responses to environmental stresses and hormones [51Fu et al., 2007]. Cold, osmotic and salt stresses, and external application of hormones such as JA, ABA, and SA, increased the expression of certain *HDA1* genes, and reduce the expression of others (51Fu et al. 2007). For example two members of the rice HDA1-class I (*HDA 702* and *HDA705*) and one member of class II (*HDA 704*) were induced by exogenous JA application. Conversely, the expression of a member of class IV (*HDA 712*) was reduced after JA treatment.

Our group has identified and characterized gene members of both HDA1 and HD2 families from barley and examined their expression during barley development and in response to stress-related hormones, such as ABA and JA [61Demetriou et al., 2009; 62Demetriou et al., 2010]. Barley *HDA1* genes (one of each class, I, II, III, and IV, respectively) were induced upon JA treatment, in agreement with the expression of their rice homologues. In addition, both *HvHDAC2-1* and *HvHDAC2-2* of the barley HD2 family, were significantly induced at 6 and 24 h after exogenous application of seedlings with JA. On the other hand, *HvHDAC2-1* showed a marked induction at 24 h after ABA treatment, whereas *HvHDAC2-2* transcript levels declined at 6 h after ABA treatment and showed no significant difference in 24 h after ABA treatment [61Demetriou et al., 2009]. In rice, the two *HD2* homologues (*HDT701)* and (*HDT702*) were also induced upon treatment with JA (51Fu et al., 2007) in accord to their barley homologues. On the contrary, whereas both rice *HD2* homologues were repressed by ABA, barley *HvHDAC2-1* and *HvHDAC2-2* showed differential responses to ABA exposure. Interestingly, the *HD2c* gene of Arabidopsis is also repressed by ABA (58Sridha and Wu, 2006). Together these results suggest common functions for some HDAC homologues among species but also possible species-specific functional diversification, in response to stress.

Histone methyltransferases(HMTs)/Histone demethylases (HDMs)

The best characterized histone methyltransferase (HMTs) genes are the ones coding for the enzymes that perform the deposition of the H3K4me3 activation mark and H3K27me3 silencing mark, respectively. These have been intensively studied both in monocots and dicots and the results of these studies have been discussed in a number of reviews [35Avramova 2009; 36Alvarez et al., 2010; 37Pontvianne et al., 2009; 38Liu et al., 2010; 39Kapazoglou et al., in press]. The Polycomb group (PcG) complex with H3K27me3 activity plays a crucial role in various stages of development, such as flowering and seed development and is composed of four subunits. Two WD40 proteins, FERTILIZATION INDEPENDENT ENDOSPERM (FIE), and MULTICOPY SUPPRESSOR OF IRA1 (MSI1) remain constant in all PcG complex variants. Depending on cell type and function the different PcG complexes contain one of the three homologues of the Drosophila E(Z) homologues, MEA, CURLY LEAF (CLF) or SWINGER (SWN), which possess the histone

methyltransferase activity, and one of the three homologues of the *Drosophila* Su(z)12 protein, EMBRYONIC FLOWER 2 (EMF2), FERTILIZATION INDEPENDENT SEED 2 (FIS2), and VERNALIZATION2 (VRN2), respectively. It was shown that Arabidopsis *msi1-cs* co-suppressor lines displayed increased tolerance to drought stress. In addition, the expression of stress- and ABA-responsive genes was up-regulated in *msi1-cs* lines suggesting that MSI1 suppresses stress-related genes in an ABA-dependent manner [[63]Alexandre et al., 2009]. A recent study implicated the Trithorax protein ATX1, performing trimethylation of H3 at lysine 4 (H3K4me3), in dehydration stress signaling both in an ABA-dependent and ABA-independent manner. *atx1* plants exhibited larger stomatal apertures, increased transpiration rates and decreased tolerance to dehydration stress. *ATX1* was shown to be required for induction of *NCED (*a gene encoding a key enzyme in ABA biosynthesis) and H3K4me3, in response to dehydration stress. By inducing *NCED3* and consequently ABA synthesis, *ATX1* exerted an effect on ABA-dependent gene expression, but it was also shown to regulated ABA-independent gene expression pathways [[64]Ding et al., 2011].

A recent study by our group characterized the *PcG* gene homologues from barley and examined their expression during seed development and in response to ABA treatment. The barley homologues, *HvE(Z)* and *HvFIE* were significantly induced at 24 hours after ABA exposure, about 4-fold and 10-fold, respectively, implying a role of PcG genes in ABA-mediated processes, such as seed development, seed dormancy, germination and abiotic stress response [[65]Kapazoglou et al., 2010]. Moreover, a gene encoding a trithorax-like H3K4 methyltransferase, *HvTX,* was also identified and characterized in barley by our group. *HvTX* transcript levels showed a marked increase by drought in a drought-tolerant barley cultivar [Papaefthimiou and Tsaftaris, in press[66]].

Histone demethylases were only recently discovered and their molecular and functional characterization is an area of active research [Kapazoglou et al., in press[39]]. In Arabidopsis, functional studies assigned a role for H3K4-specific demethylases as regulators of flowering time by deactivating the flowering repressor gene *FLC* and promoting flowering [[67]]. In rice, a jmjC domain-containing gene encoding a H3K9 demethylase, JMJ706, was found to be required for floral organ development[[68]]. Reports describing a putative role of HDMs in abiotic stress are anticipated. In the cereal crop barley, one putative plant-specific PKDM7 subfamily histone demethylase was characterised and was shown to be significantly induced by drought stress [[69]Papaefthimiou and Tsaftaris, in revision].

3. ATP-dependent chromatin remodeling factors

The SWI/SNF (switch/sucrose non-fermenting) is a multisubunit assembly with DNA-dependent ATPase activity that is implicated in alteration of chromatin structure and subsequent changes in gene expression [[70]Schwabish and Stuhl, 2007]. An SNF-type putative remodeling gene was shown to be expressed in a desiccation- and ABA-dependent manner in pea [[71]Rios et al., 2007]. AtCHR12, a SNF/Brahma (BRM)-type chromatin remodeling factor, has been implicated as a negative regulator in the temporary growth arrest caused by drought and heat stress, in Arabidopsis [[72]Mlynarova et al., 2007]. Overexpression of *AtCHR12* resulted in growth arrest of primary buds and reduced growth of primary stems under drought and heat stress. On the contrary, in *atchr12* knockout mutants growth arrest was decreased as compared to wild type plants under stress. In another report it was shown that SWI3B, a subunit of a SWI/SNF complex in Arabidopsis, interacts with HAB1, (a

phosphatase 2C), which is a negative regulator of ABA signaling [73Saez et al., 2008]. *swi3b* mutant seedlings exposed to external ABA exhibited reduced sensitivity to ABA-mediated inhibition of seed germination and growth and reduced expression of ABA-responsive genes like *RD29B* and *RAB18* [73Saez et al., 2008]. Furthermore, ChIP assays showed that the interaction of HAB1 with *RD29B* and *RAB18* promoters was abolished by ABA, suggesting that HAB1 modulates the ABA response through regulation of a SWI/SNF complex.

Molecular and functional characterization of chromatin remodeling factors in cereals is scarce. In one study it was shown that ChIP assays conducted with maize leaf nuclei, detected an enrichment for SWI2/SNF2 at target genes after UV-B treatment of maize plants, implying involvement of chromatin remodelling factors in abiotic stress responses [44Casati et al., 2008]. It is expected that by exploiting the data from the completed rice, maize and recently Brachypodium genomes, additional studies on chromatin remodeling and its association with abiotic stress in cereals will soon be reported.

4. DNA methylation/demethylation

DNA methylation is a critical epigenetic modification which is established and maintained by multiple interacting cellular mechanisms. Cytosine methylation in plants is found predominately in a symmetrical CG dinucleotide site. However unlike animals, it also occurs at CHG and asymmetric CHH sites (where H is A, C, or T). A dynamic interplay between methylation and demethylation accomplished through specific enzymes, is critical for proper cellular regulation during plant development. DNA methylation is carried out by "de novo" and "maintenance" DNA methyltransferases (MTases), and in most cases results in gene silencing although the opposite has been also observed [7Law and Jacobsen, 2010; 74Macarevich et al., 2008; 75Shibuya 2009]. A number of reports have demonstrated that DNA methylation may be employed by plants to regulated gene expression as a response to abiotic stresses.

An early study in maize had shown that cold stress induced the expression of the *ZmMI1* gene (a retrotransposon-like gene) and this correlated with reduction in nucleosomal DNA methylation [76Steward et al., 2002]. Studies of F1 hybrids and their parents in maize revealed that under dense planting (a stressful condition), parents accumulated more DNA methylation sites than their hybrids which resist to DNA methylation changes [77Kovacevic et al., 2005; 78Tani et al., 2005; and reviewed in 79Tsaftaris et al., 2008]. Another report in tobacco showed that a methyltransferase (met1) mutant, exhibited demethylation of genomic regions that were associated with the expression of a large number of drought-related genes [80Wada et al., 2004]. Moreover, tobacco plants exposed to high salt, cold and aluminum displayed changes in the methylation pattern of a gene encoding glycerophosphodiesterase-like protein (NtGPDL) and known to be induced in response to aluminum stress, as compared to nonstressed plants [81Choi and Sano, 2007]. CG sites within the coding region were selectively demethylated suggesting that abiotic stress caused gene activation by changing the DNA methylation status of the particular genomic locus. A recent study exploring the genome-wide DNA methylation status of two rice cultivars with different tolerance to drought, revealed significant differences in the methylation patterns between the two genomes [82Wang et al., 2011]. In particular, a drought-tolerant line DK151 and its drought-sensitive parent, IR64, were anaadapatationlyzed by methylation-sensitive amplified polymorphism analysis (MSAP) under drought stress and no stress conditions.

DNA methylation/demethylation changes were induced under drought conditions in a developmental and tissue specific manner and they accounted for 12.1% of the total site-specific methylation differences between the two lines. Notably 70% drought-induced methylation changes were reversed after recovery, and 29% remained unaltered. These observations suggest that DNA methylation changes play a role in the response of rice to dehydration conditions probably by activating or deactivating stress-responsive genes and leading to adaptation to drought conditions [82Wang et al., 2011]. MSAP was also used recently in wheat, to assess DNA methylation changes upon salt stress in two cultivars with different tolerance to salt. Upon high salinity conditions DNA methylation alterations were observed in both cultivars which might be associated with the response and adaptation of wheat to salt stress [83Zhong et al., 2009].

Unlike the well characterized histone modification enzymes HATs, HDACs and HMTs, little is known regarding DNA methyltransferases and demethylases in association to stress. Ten putative DNA methyltransferases were characterized in rice and their expression examined in different developmental stages and under abiotic stress. *OsCMT2* was found to be induced by cold and high salinity but not by drought. Conversely, *OsCMT3* showed approximately a six- and four-fold reduction in mRNA accumulation in rice seedlings subjected to high salt and dehydration conditions, respectively [84Sharma et al., 2009]. In a recent study, the gene encoding the Arabidopsis DNA glycosylase ROS1 (REPRESSOR OF SILENCING 1)-now known as DML3 (DEMETER-LIKE protein 3) and involved in DNA demethylation-was indirectly implicated in the response to abiotic stress, as it was shown to be the target of the stress-responsive miRNA402 [85Kim et al., 2010].

5. Small RNAs

Four major types of small RNAs have been identified in plants, namely, micro RNAs (miRNAs), transacting small interfering RNAs (ta-siRNAs), natural-antisense siRNAs (nat-siRNAs), and heterochromatic (hc-RNAs) siRNAs. Hc-siRNAs direct methylation of DNA sequences complementary to the siRNAs in a process known as RNA-directed DNA methylation (RdDM) and lead to gene silencing [8Chapman and Carrington, 2007; 9Henderson and Jacobsen. 2007]. MiRNAs, ta-siRNAs, and nat-siRNas function predominately at the post-transcriptional level through mRNA degradation and/or translational inhibition resulting in gene silencing, and miRNAs have been shown to also regulate gene expression through DNA methylation [86Wu et al., 2009; 87Khraiwesh et al., 2010].

Small RNAs have essential functions in many aspects of plant growth and development [Liu et al., 2005; 88Jones-Rhoades et al, 2006; 12Voinnet 2009; Mallory and Vaucheret, 2006; 89Chen, 2009]. Furthermore, small RNAs have been shown to play key roles in the regulation of phytohormone signaling and the response to a variety of abiotic stresses [90Sunkar and Zhu 2004; 91Sunkar et al., 2007; 92Voinnet 2008; Liu and Chen, 2009; 93Covarrubias and Reyes, 2010].

Locus-specific studies as well as large-scale transcriptome analyses have revealed numerous miRNAs that are conserved across species and are responsive to a broad spectrum of stresses. In the last several years the development of high-throughput sequencing technology has allowed for the discovery of ever more miRNAs including very low abundance or species-specific miRNAs. In this way a growing number of small RNAs has been detected that respond to abiotic (as well as biotic) stress both in dicots and monocots.

In Arabidopsis, stress-related miRNAs were first detected in a library generated from small RNAs from seedlings exposed to various stresses ([94]Sunkar and Zhu, 2004). For example miR393, miR397b, and miR402 were found to be induced upon cold, drought and high salinity conditions as well as by ABA treatment. Follow-up studies with miR402 showed that miR402 overexpressing plants displayed reduced transcripts of the DNA demethylase DML3, implying miRNA-guided control through down-regulation of a DNA demethylase [[85]Kim et al., 2010].

An siRNA derived from a pair of natural *cis*-antisense transcript composed of *PYRROLINE-5-CARBOXYLATE DEHYDROGENASE(P5CDH)* (sense), a stress-related gene, and *SRO5* (antisense), a gene of unknown function, generates two types of siRNAs, 24-nt siRNA and 21-nt siRNA. These were found to down-regulate *P5CDH* by sequential cleavage of *P5CDH* mRNA after salt treatment leading to accumulation of the osmoprotectant proline and increased tolerance to salt stress [[95]Borsani et al., 2005]. Stress- or ABA-inducible sense and antisense transcripts were also detected in the stress-inducible gene loci, *RD29A* and *CYP707A1* [[96]Matsui et al., 2008]. Transcriptome microarray analysis revealed numerous other miRNAs involved in abiotic stress both in Arabidopsis and poplar [[97]Liu et al., 2008; [98]Lu et al., 2008]. Conserved miRNAs, such as miR397 and miR169, were up-regulated in both species under cold conditions, and species-specific stress responsive miRNAs were also detected.

MiRNA responsiveness to various abiotic stress factors has been demonstrated in cereals such as rice, wheat, maize and the model-plant of cereals, Brachypodium. For example drought and high salinity stress were found to induce several miRNAs in rice as determined by microarray analysis [[99]Zhao et al., 2009]. MiR169g was shown to be up-regulated in rice roots and shoots upon dehydration. Interestingly, the promoter of the miR169g gene was found to contain two dehydration responsive elements (DRE). Similar to miR169g, the rice miR169n gene was found to be induced at conditions of high salinity. A cis-acting ABA responsive element (ABRE) resides within the promoter of rice miR169n implying an ABA-mediated response to stress [[99]Zhao et al., 2009]. Notably, both miRNAs target a transcription factor, NF-YA, that has been shown to be down-regulated upon drought conditions [[100]Stephenson et al., 2007]. Recently, genome-wide profiling of miRNAs in rice revealed 29 novel miRNAs that were differentially expressed (11 down-regulated miRNAs and eight up-regulated) under drought [[25]Sunkar et al., 2008; [101]Zhou et al., 2010].

[102]Kantar et al. (2010), identified 28 new miRNAs in barley, of which Hvu-MIR156, Hvu-MIR166, Hvu-MIR171, and Hvu-MIR408 were shown to be induced under dehydration conditions. Microarray analysis in maize demonstrated that 34 miRNAs from 13 plant miRNA families exhibited substantial changes in expression after drought treatment of seedlings [Wei et al., 2009103]. MiR474 which targets a gene encoding proline dehydrogenase (PDH), an enzyme involved in the degradation of proline, was found to be up-regulated upon dehydration conditions. Proline is known to accumulate in plants as a protective mechanism against drought stress. Upon drought stress miR474 transcripts were increased, whereas PDH accumulation was reduced, suggestive of a miR474-dependent mechanism in regulating proline content under drought conditions in maize. Conversely, the expression of other maize miRNAs such as miR168, miR528, and miR167 was decreased and this probably resulted in increased expression of their target genes *MAPK (MITOGEN ACTIVATED PROTEIN KINASE)*, *POD (PEROXIDASE)*, and *PLD (PHOSPHOLIPASE D)*, respectively. Interestingly, these genes contain an ABA responsive element and are involved in the ABA-induced stomatal movement and antioxidant defense in maize [Wei et al., 2009[103]].

Cold stress has also been shown to have a significant effect in the expression of a number of different miRNAs in cereals. Microarray analysis identified 18 rice miRNAs that were differentially expressed upon cold treatment of rice seedlings [104Lv et al., 2010]. 12 miRNAs corresponding to 10 different families exhibited significant down-regulation and 6 miRNAs corresponding to five families exhibited substantial up-regulation under cold. Four down-regulated rice miRNAs (miR1435, miR1876, miR1320, miR1884) were not present in Arabidopsis implying species-specific miRNas functions in the response to cold-stress. Six conserved families (miR156, miR166, miR169, miR171, miR319, miR444) are known to target genes encoding transcriptional factors such as homeodomain-leucine zipper proteins, scarecrow-like proteins, TCP family transcription factors and MADS-box proteins [Lu et al., 2008; Zhao et al., 2009]. The targets of rice miR319a/b and miR171a, were predicted to be the genes Os01g59660 and Os04g46860, respectively. Os01g59660 and Os04g46860 were induced by cold, whereas their cognate miRNAs were found to be down-regulated by cold. This inverse correlation between the expression of the miRNAs and their targets and the fact that the targets were validated by 5'RACE assays, strongly suggests miRNA-regulated responsiveness to cold stress [104Lv et al., 2010]. Interestingly, rice miR444 which is also down-regulated by cold-stress, targets two MADS-box proteins, MADS57 and MADS27 [Lu et al., 2008] which have been shown previously to be up-regulated under cold conditions [105Arora et al., 2007]. Most cold-responsive miRNAs were found to harbor cis-acting hormone-responsive elements in their 5'upstream regions, such as ABRE, and GARE (Gibberellin responsive element). For example, an ABRE element and two GARE elements were detected within the miR319 promoter implying ABA-mediated regulation of gene expression. In support to this a recent study showed that miR319 is down-regulated by ABA and up-regulated by GA, and a large number of other rice miRNAs are either induced or down-regulated by ABA and GA [106Liu et al., 2009].

High throughput sequencing technology using the Solexa platform, uncovered 129 putative novel miRNAs in the model plant Brachypodium. 25 of the novel miRNAs as well as 3 conserved miRNAs (miR169e, miR172b and miR397) displayed significant alterations in gene expression in response to cold stress [23Zhang et al., 2009]. A subset of the novel cold-responsive miRNAs was found to be monocot-specific and another subset Brachypodium-specific. MiR169e , miR172 and miR397 and six of the novel predicted miRNAs were up-regulated under cold, whereas 19 novel miRNAs were down-regulated. Interestingly, miR397 is predicted to target laccases, enzymes involved in lignin biosynthesis and cell wall structure maintenance.

A recent study described the identification of a set of miRNAs from wheat that responded to heat stress as well as to the biotic-stress conditions of powdery mildew infection [107Xin et al, 2010]. Furthermore, by interrogating the recently deep-sequenced small RNA transcriptome of bread wheat, Yao et al. 2010[29] identified a set of small non-coding RNAs with differential responses in a variety of stress conditions. For example siRNA 002061_0636_3054.1 shows down-regulation under conditions of increased heat, salinity and dehydration, whereas siRNA 005047_0654_19041.1 is substantially induced by cold.

SiRNAs have been also implicated in abiotic stress response in rice [108Yan et al., 2011]. Rice siR441 and siR446 accumulation was down-regulated by cold, drought, high salinity and by ABA treatment. Functional analysis showed that siR441 and siR446 knockdown mutants were more sensitive to drought, cold or salt treatment than the wild type, suggesting a role for siRNAs in rice tolerance to abiotic stress. The validated target of siR441 and siR446, *MAIF1*(encoding an F-box protein), was previously shown to be up regulated under abiotic

stress conditions. In addition, transgenic rice plants with decreased accumulation of siR441 and siR446 had the same phenotype as *MAIF1* overexpressing plants [108Yan et al., 2010]. Together these observations point to a role for rice siR441 and siR446 in abiotic stress response through regulation of *MAIF1*.

Genome-wide studies of intraspecific hybrids and their parents, in Arabidopsis, have revealed major differences in the 24-nt siRNA levels between the two genomes which resulted in alterations in global DNA methylation and gene expression [109Groszman et al., 2011]. Hybrid vigor is characterized by the superior performance of a hybrid over its parents in various traits, including stress tolerance, and this suggests that siRNA pathways may be associated with abiotic stress response in this phenomenon.

Finally, a recent report showed that siRNA biogenesis is crucial for protection against transgenerational retrotransposition under heat stress, in Arabidopsis [110Ito et al., 2011]. It is likely that such stress-related siRNA/retrotransposon effects will be revealed for cereal genomes as well.

6. Transgenerational stress memory

Adverse environmental conditions may induce changes in the epigenetic state of genes which can be inherited over successive generations and these could play a role in stress adaptation [111Paszkowski and Grossniklaus, in press].

Exposure to stress can result in changes in DNA methylation patterns and genome instability. Studies on *Arabidopsis and Pinus silvestris* growing in the vicinity of the Chernobyl reactor area suggested an association between increased global genome methylation with genome stability and stress tolerance in response to irradiation [112Kovalchuk et al., 2003; 1132004]. An association between transgenerational changes in DNA methylation and stress tolerance was also reported in the progeny of plants exposed to different abiotic stresses [114Boyko et al. 2010]. Arabidopsis plants were exposed to a wide spectrum of abiotic stresses including high salinity, UV-C, cold and heat as well as biotic stress. This resulted in higher homologous recombination frequency, increased global DNA methylation and higher stress tolerance in the untreated progeny. Moreover, in mutants defective in *DICER-like* genes, important for siRNA biosynthesis pathways, stress-induced homologous recombination frequency, DNA methylation and stress tolerance were impaired. These results suggested that stress-induced transgenerational responses require DNA methylation and the function of siRNA silencing pathways.

The significance of induced genome changes in adaptation was examined also in rice [115Akimoto et al., 2007]. Rice seeds were treated with 5-aza-deoxycytidine (inhibitor of cytosine methylation) and progeny after ten generations was screened to identify changes in DNA methylation by the MSAP and bisulfite assays. In one of the tested lines, line-2, DNA methylation was completely abolished in the gene coding region for the *Xa21G* gene encoding the Xa21-like protein. In wild type plants the *XA21G* promoter was methylated and there was no detectable expression of *Xa21G*, whereas in the line-2, *Xa21G* was expressed constitutively and the line was resistant to the pathogen race *Xanthomonas oryzae* pv. *oryzae*, race PR. These results suggested that DNA methylation can be stably inherited and maybe associated with the plants adaptation to stressful environments.

With the rapid progress in epigenetic research it is expected that further studies will emerge on the association of epigenetic states and transgenerational stress memory in more crop species.

7. Conclusions

Great progress in the research of epigenetic regulation in response to abiotic stress has been accomplished in the last several years, especially in the model plant Arabidopsis. Changes in histone modifications and changes in the expression of genes encoding histone modifying enzymes, as well as changes in DNA methylation patterns and the effect of small RNAs have been shown to play critical roles in the response to abiotic stress at a gene-specific and genome-wide level. Similar studies have been performed in cereals and a growing number of reports on the epigenetic regulation during cereal plant development and in response to abiotic stress have accumulated. However, plenty more efforts are still required in order to fully characterize and understand this process. The completion of the two cereal genomes, rice and maize, and of the cereal/grass-model plant Brachypodium, as well as the rapid progress in the sequencing of wheat and barley, will contribute significantly to this endeavor. The detailed study of both the genetic and epigenetic components of this complex process is necessary to comprehend the molecular aspects of the abiotic stress response. Furthermore, understanding the molecular mechanisms underlying the association of epigenetic regulation and transgenerational stress memory will help us in establishing the potential adaptive significance of this process and could have significant implications in agriculture. Considering that cereals represent approximately 50% of total caloric intake worldwide (www.fao.org) and in view of the upcoming adverse changes of the global climate it is vital to delineate the molecular mechanisms by which such agronomically important crops manage to cope under conditions of stress. This could have important ramifications for agriculture as it would enable the generation of improved varieties with increased stress tolerance.

8. References

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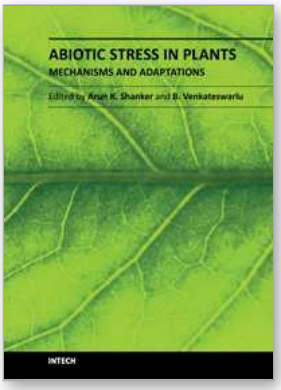

Abiotic Stress in Plants - Mechanisms and Adaptations

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World population is growing at an alarming rate and is anticipated to reach about six billion by the end of year 2050. On the other hand, agricultural productivity is not increasing at a required rate to keep up with the food demand. The reasons for this are water shortages, depleting soil fertility and mainly various abiotic stresses. The fast pace at which developments and novel findings that are recently taking place in the cutting edge areas of molecular biology and basic genetics, have reinforced and augmented the efficiency of science outputs in dealing with plant abiotic stresses. In depth understanding of the stresses and their effects on plants is of paramount importance to evolve effective strategies to counter them. This book is broadly dived into sections on the stresses, their mechanisms and tolerance, genetics and adaptation, and focuses on the mechanic aspects in addition to touching some adaptation features. The chief objective of the book hence is to deliver state of the art information for comprehending the nature of abiotic stress in plants. We attempted here to present a judicious mixture of outlooks in order to interest workers in all areas of plant sciences.

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