ABA-mediated Epigenetic Processes in Plant Development and Stress Responses
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Running Title:

ABA Regulated Epigenetic Processes
Abstract
Abscisic acid (ABA) regulates diverse plant processes, growth and development under non-stress conditions and plays a pivotal role in abiotic stress tolerance. Although ABA-regulated genetic processes are well known, recent discoveries reveal that epigenetic processes are an integral part of ABA-regulated processes. Epigenetic mechanisms, namely histone modifications and cytosine DNA methylation, induce modification of the genome, giving rise to epigenomes, which add diversity and complexity to the genome of organisms. Histone monoubiquitination appears to regulate ABA levels in developing seeds through histone H2B monoubiquitination. ABA and H2B ubiquitination-dependent chromatin remodeling regulate seed dormancy. Transcription factor networks necessary for seed maturation are repressed by histone deacetylases (HDACs)-dependent and PIKLE chromatin remodeling complexes (CRCs), while ABA induces the expression of these genes directly or through repression of HDACs. Abiotic stress-induced ABA regulates stomatal response and stress-responsive gene expression through HDACs and HOS15-dependent histone deacetylation as well as through ATP-dependent SWI/SNF CRC. ABA also probably regulates abiotic stress response through DNA methylation and siRNA pathways. Further studies on ABA-regulated epigenome will be of immense use to understand the plant development, stress adaptation, and stress memory.

Key words: abscisic acid, chromatin remodeling, DNA methylation, histone deacetylases, abiotic stress memory

Introduction
Abscisic Acid (ABA) regulates plant growth and development such as germination, lateral root development, seedling growth, seed development, seed dormancy, transition from vegetative to reproductive phase, and abiotic stress tolerance. Diverse roles of ABA in plants suggest the existence of multiple receptors and signal transduction pathways. Only recently three possible ABA-receptors, namely, FCA (Flowering time Control protein A) (Razem et al., 2006), ABAR (ABA Receptor) similar to H subunit of Mg-chelatase (CHLH) (Shen et al., 2006) and G protein-coupled receptor (GCR2) (Liu et al., 2007a) have been identified. Although ABA signaling pathways are not yet thoroughly...
understood, it is very well established that most of the ABA responses (baring the quick stomatal closure response) are regulated by ABA-mediated transcriptional regulation, which are extensively reviewed earlier (Schroeder et al., 2001; Finkelstein et al., 2002; Zhu 2002; Chinnusamy et al., 2004; Nambara and Marion-Poll 2005; Yamaguchi-Shinozaki and Shinozaki 2006; Wasilewska et al., 2008). Recent discoveries reveal that besides genetic regulation, epigenetic regulation plays a key role in ABA-mediated plant processes. This review will briefly introduce the epigenetic processes, namely, histone modifications and cytosine DNA methylation, and further focus on the role of epigenetic processes in ABA-mediated developmental and stress response processes.

1. Epigenetic Processes

Epigenetics is defined as the study of changes in gene function that are mitotically and/or meiotically heritable, and that do not entail a change in DNA sequence (Wu and Morris 2001). Later, the definition included chromosomal marks that are transient as well as heritable (Bird 2007). Epigenetic modifications play pivotal roles in genomic imprinting (Feil and Berger 2007), paramutation (Alleman et al., 2006), defense against transposon proliferation (Vaucheret and Fagard 2001) and transcriptional gene silencing (Gong et al., 2002). Besides these phenomena, genomes are responsive to, and regulated by developmental and environmental cues through epigenetic processes.

1.1 Histone Code

The basic unit of eukaryotic chromosome is the nucleosome, which consists of a histone-core complex (H2A, H2B, H3 and H4) wrapped around by 146 bp DNA and the linker histone H1 associated with the linker DNA (8 to 114 bp). Nucleosome structure depends upon the histone variants and post-translational modifications in the N-terminal tails of core histones. Arabidopsis genome encodes about 50 genes for histones (5 H1, 13 H2A, 11 H2B, 13 H3 and 8 H4). The N-tails of the core histones offer about 240 sites for posttranslational modifications such as acetylation (Peterson and Laniel 2005), methylation (Shilatifard, 2006), phosphorylation (Houben et al., 2007), biotinylation (Camporeale et al., 2007), ubiquitination (Sridhar et al., 2007), sumoylation (Nathan et al., 2006) and ADP-ribosylation (Hassa et al., 2006). Histone acetylation, phosphorylation, and ubiquitination activate transcription (Peterson and Laniel 2005;
Houben et al., 2007; Sridhar et al., 2007), while biotinylation and sumoylation repress gene expression (Nathan et al., 2006; Camporeale et al., 2007). Dimethylation of H3 (K9 and K36) represses transcription, while trimethylation of H3K4 activates transcription (Zhang et al., 2007). Specific combinations histone variants and core histones N-tail post-translational modifications called "histone code" offer enormous combinatorial possibilities for nucleosome assemblies. Histone code plays a key role in chromatin structure and affinity for chromatin-associated proteins, and thus regulates the expression of genetic code (Jenuwein and Allis 2001; Fuchs et al., 2006). Chromatin modification is induced by histone modification-dependent chromatin remodeling complexes (CRCs) and ATP-dependent CRCs (SWITCH/ SUCROSE NONFERMENTING, SWI/SNF complex) that non-covalently modify histones in the nucleosome (Fransz and de Jong 2002).

1.2 DNA Methylation

Cytosine (5') methylation of DNA is the ubiquitous mechanism of heritable epigenetic modification. DNA methylation maintains the methylated loci at repressed state. In plants, asymmetric methylation (mCpHpH) is re-established after each mitosis cell division, whereas symmetric methylation (mCpG and mCpNpG) can be maintained across mitosis and meiosis divisions (Martienssen and Colot 2001). The de novo DNA methyltransferases catalyze new cytosine methylation, while maintenance DNA methyltransferases propagates the symmetric methylation marks on the parental DNA (Chan et al., 2005). The Arabidopsis bifunctional DNA glycosylase/lyase REPRESSOR OF SILENCING-1 (ROS1) actively removes DNA methylation by a base-excision repair mechanism (Gong et al., 2002; Agius et al., 2006), and thus has a key role in the plasticity of the epigenome (Zhu et al., 2007b). Arabidopsis DEMETER (DME), a protein similar to ROS1, is necessary for endosperm gene imprinting and seed viability in Arabidopsis (Choi et al., 2002). Post-translational modifications of histones and DNA methylation determine chromatin modeling and remodeling. These modifications can transmit epigenetic memory within one generation and across generations (Khorasanizadeh, 2004; Vaillant and Paszkowski, 2007).

2. ABA-mediated Epigenetic Processes in Seeds and Seedlings
Genetic analyses of ABA deficient and ABA response mutants showed that ABA plays pivotal roles in storage reserve accumulation, desiccation tolerance, and dormancy of seeds and germination arrest. During seed development, two peaks of ABA accumulation were observed. Maternally derived early peak of ABA is required for embryo maturation and prevention of vivipary. The embryo synthesizes the second peak of ABA accumulation in seed and this ABA appears to regulate dormancy, seed reserve accumulation and desiccation tolerance. Gene expression during seed development and germination inhibition are regulated by ABA through networks of transcription factors belonging to B3 domain (ABI3, ABA INSENSITIVE3/VP1, VIVIPAROUS1; LEC2, LEAFY-COTYLEDON2; FUS3 FUSCA3), APETELA2 (ABI4), bZIP (ABI5) and HAP3 subunit of CCAAT binding factor (LEC1) (Frankelstein et al., 2002).

2.1 Seed Maturation
ABA plays a central role in accumulation of storage reserve and acquisition of desiccation tolerance during seed maturation. Seed storage protein accumulation requires a B3 transcription factor such as ALF (ABI3-like factor, Phaseolus vulgaris), ABI3 (in Arabidopsis), or VP1 (in Zea mays). The phaseolin (phas, encoding a major seed storage protein) gene is repressed in vegetative tissues and chromatin remodeling activates transcription of phas in seeds. PvALF1 transcription factor activates the ABA-dependent seed-specific expression of phas. Repression of phas expression is related to the deacetylated chromatin structure on phas promoter in leaves. DNase I foot printing assay revealed that the TATA boxes of phas are protected in the presence of both PvALF and ABA. ABA-induced expression of PvALF and the remodeling of chromatin architecture over the TATA box region of the phas promoter are required for phas expression (Li et al., 1999). Chromatin immunoprecipitation (ChIP) assay revealed that PvALF potentiates chromatin for transcription by mediating acetylation of H3-K9 and H4-K12 through histone acetyltransferases (HATs), whereas ABA has been shown to induce acetylation of H3-K14 and methylation of H3-K4 (Ng et al., 2006). Thus, chromatin remodeling in a euchromatin region play a crucial role in ABA-induced gene expression.

Arabidopsis B3 domain transcription factors such as LEC2, FUS3, and ABI3 activate genes involved in accumulation of storage proteins and lipids in the embryo during seed maturation. After completion of seed maturation, these genes are repressed in
matured seeds and vegetative tissues. *Arabidopsis pkl (pikle)* and *val (vp1/abi3-like)* mutants exhibited derepression of embryonic genes in germinating seedlings (Ogas et al., 1999; Suzuki et al., 2007). The *PIKLE (PKL)* gene encodes a putative SWI/SNF-class chromatin-remodeling factor of the CHD3 type (harboring a Chromodomain, a SNF2-related helicase/ATPase domain, a DNA binding domain and a PHD zinc finger). *VALs* encode B3 proteins associated with chromatin factors. *val1 val2* double mutant seedlings showed embryonic seedling phenotype, and upregulation of *LEC1, ABI3*, and *FUS3* genes. It is suggested that VAL proteins repress the target genes by recruiting a chromatin-remodeling complex that includes PKL and related CHD3 proteins (Suzuki et al., 2007). Histone acetylation dependent chromatin remodeling also appears to regulate seed development as plant-specific histone deacetylases (HDACs) such as HD2A, HD2B, and HD2C have been shown to express in ovules, embryos, shoot apical meristems, and primary leaves and strongly induced during somatic embryogenesis in *Arabidopsis* (Zhou et al., 2004).

### 2.2 Seed Dormancy

In natural ecosystem, plants complete their life cycle in favorable season and the seeds remain dormant during the unfavorable season. Thus, seed dormancy is crucial to avoid vivipary (pre-harvest germination of seeds on mother plant) and germination of seeds under unfavorable conditions (Bewley et al., 1997). Besides, successful agriculture also requires seed dormancy, as seeds harvested in the previous season needs to be stored till the next season. Mutations that impair ABA biosynthesis (*aba*) and mode of action (*abi1 to abi5, era1, sad1, abh1*) also impair seed dormancy and germination under stress conditions. Molecular genetic analyses established the pivotal role of ABA, the original “dormin”, in inducing seed dormancy and prevention of germination. ABA is necessary for seed dormancy in many plants (Frankelstein et al., 2002) and tuber dormancy in potato (Suttle and Hultstrand 1994). However the mechanisms of dormancy and germination arrest are poorly understood. The ABA-mediated chromatin remodeling processes regulate some of the processes involved in seed dormancy and inhibition of germination under unfavorable conditions.

Treatment of potato tubers with bromoethane (BE, artificial breaker of dormancy) resulted in a decline of meristem ABA content due to reduction in the expression of
StNCED (9-cis-epoxycarotenoid dioxygenase) and enhanced expression of StCYP707A1 and StCYP707A2 (ABA 8’-hydroxylases that catalyze inactivation of ABA) genes (Destefano-Beltrán et al., 2006). Expression pattern of these genes also showed similar pattern during ripening process in Arabidopsis seeds. Genetic analysis of Arabidopsis mutant, reduced dormancy4 (rdo4), which exhibits reduced seed dormancy, revealed a possible role of chromatin remodeling in seed dormancy through regulation ABA metabolism and response genes (Liu et al., 2007b).

The RDO4, was named later as HISTONE MONOUBIQUITINATION1 (HUB1), encodes a C3HC4 RING finger E3 ligase, which catalyzes histone H2B monoubiquitination. Mutation in HUB2, a homolog of HUB1, also reduced seed dormancy in Arabidopsis. hub1 and hub2 mutants are deficient in ubiquitinated H2B. Dormancy related genes which are involved in ABA metabolism and response such as NCED9 and ABI4 showed reduced expression, while CYP707A2 showed enhanced expression in the freshly harvested seeds of hub1 mutant as compared with wild-type. Besides these genes, expression of DOG1 (DELAY OF GERMINATION 1), ATS2 (a caleosin-like protein) and PER1 (similar to the peroxiredoxin family of antioxidants) were reduced in freshly harvested seeds of hub1 mutant. These expression patterns were similar to that of wild type after-ripened seeds (Liu et al., 2007). Histone H2B monoubiquitination is associated with activation of gene transcription and deubiquitination is linked to repression (Sridhar et al., 2007). Hence, chromatin remodeling through HUB1 and HUB2 mediated H2B monoubiquitination plays a key role in seed dormancy (Liu et al., 2007) probably by regulating ABA level in seeds, ABA sensitivity and other mechanisms.

2.3 Germination Inhibition

Osmotic stress and salt concentration will be high during the non-rainy season and will decrease during the rainy season in salt affected soils. In nature scanty rainfall frequently occurs in non-rainy seasons with ensuing longer drought spells. If seeds germinate under these unfavorable conditions, then the chance for their survival is less. Hence, plants have impregnated adaptive mechanism in seeds to prevent germination, if osmotic stress occurs during the initial processes of germination. Exogenous application of ABA or osmotic stress within with in 48 h of imbibition can arrest or delay germination of
Arabidopsis seeds. Germination arrest is mediated by induction and maintenance of ABI3 and ABI5 transcription factors expression. Chromatin remodeling is crucial for this germination arrest mediated by ABA and osmotic stresses. In Arabidopsis, The PKL (SWI/SNF type chromatin remodeling factor) expression is induced by imbibition and it mediates repression of embryonic traits during germination (Henderson et al., 2004; Li et al., 2005). During germination LEAFY COTYLEDON1, (LEC1, a transcriptional regulator that promotes embryonic identity) and FUSCA3 (FUS3) are repressed in wild type, while pkl mutants showed expression of LEC1 and FUS3 upon seed imbibition (Ogas et al., 1999). ABA has been shown to have a modest effect on the penetrance of pklle root phenotype (Henderson et al., 2004). Further, pkl mutants exhibit hypersensitive germination responses to ABA, and persistent high expression of ABI3 and ABI5 on ABA induction. ChIP studies comparing wild-type and pkl mutant seeds treated with or without ABA revealed ABA-treated pkl mutant seeds with 2- to 2.5-fold lower H3-K9 and H3-K27 methylation levels at ABI3 and ABI5 promoters than wild-type plants. Thus, chromatin remodeling mediated by PKL represses ABI3, ABI5 and some late embryogenesis abundant genes expression after imbibition to promote germination (Perruc et al., 2007).

Besides the histone methylation, histone deacetylation is also involved in ABA sensitivity during germination. Some of the histone deacetylases (HDACs) are induced by ABA in Arabidopsis (Sridha and Wu 2006) and rice (Fu et al., 2007). Impairment of expression of AtERF7 (APETALA2/EREBP-type transcription factor) showed a role for HDA19 in ABA sensitivity during seed germination. Arabidopsis ERF7 interacts with a global corepressor of transcription, AtSin3, which in turn interacts with HDA19. HDA19 and AtSin3 enhance the AtERF7 mediated transcriptional repression. RNAi mediated suppression of AtERF7 and AtSin3 in transgenic plants resulted in increased sensitivity to ABA during germination and seedling growth. ABA signaling appears to repress the genes through histone acetylation (Song et al., 2005). Transgenic HDA6-RNAi repression line treated with the HDAC inhibitor (trichostatin A) showed growth arrest and elevated expression of LEC1, FUS3, and ABI3 during germination. lec1, fus3, and abi3 mutants suppressed the growth-arrest phenotype of the HDA6-RNAi repression plants. Double repression line of HDA6/HDA19 displayed arrested growth after germination and the
formation of embryo-like structures. These results suggest that HDA6 and HDA19 redundantly regulate the repression of embryonic properties and growth arrest during germination (Tanaka et al., 2008). In *Arabidopsis*, HDA6 has also been implicated in transgene silencing and the regulation of ribosomal RNA transcription (Probst et al., 2004; Earley et al., 2006). These lines of evidence suggest that histone deacetylation plays important roles in induction of embryonic genes and germination inhibition under unfavorable environments.

3. Epigenetic Processes in ABA-mediated Abiotic Stress Responses

The prominent role of ABA in abiotic stress adaptation of plants is well known. Stomatal regulation by ABA is crucial for regulation of transpiration and avoiding water-deficit stress in plants. ABA mediated regulation of ion channels in guard cells is crucial for stomatal closure under unfavorable conditions. Calcium, protein kinases and phosphatases, and membrane trafficking components have been implicated in ABA signaling in guard cells (Schroeder et al., 2001). Similarly, calcium, protein kinases, protein phosphatases and transcription factor networks regulate expression of ABA-responsive genes and stress adaptation (Finkelstein et al., 2002; Zhu 2002; Chinnusamy et al., 2004; Yamaguchi Shinozaki and Shinozaki 2006; Wasilewska et al., 2008).

3.1 Histone Code Regulates Stomatal Response

Histone variants offer a means to alter histone code. In tomato, the linker histone H1 variant, H1-S, was induced by drought through an ABA-dependent pathway (Bray et al., 1999; Scippa et al., 2000). This H1-S might be positively involved in drought tolerance by controlling transpiration as H1-S antisense tomato transgenic plants showed about 37% higher stomatal conductance, transpiration and photosynthetic rate than the wild type plants (Scippa et al., 2004). ABA might mediate stomatal response by chromatin remodeling through change in histone acetylation status at certain loci. Histone deacetylation also appears to regulate guard cells. *Arabidopsis* ERF7 interacts with AtSin3 and HDA19 to repress transcription of some genes. Transgenic *Arabidopsis* plants overexpressing *AtERF7* show reduced sensitivity of guard cells to ABA, and increased transpirational water loss (Song et al., 2005). Further, ABA represses the expression of *AtHD2C*, and overexpression of *AtHD2C* resulted in lower transpiration rate than that of
the wild type plants (Sridha and Wu 2006). These results show that ABA-induced stomatal response mediated by chromatin remodeling plays a crucial role in stress tolerance.

3.2 ABA-mediated Stress-Responsive Gene Expression

Histone deacetylation results in a non-permissive chromatin conformation that represses transcription. Arabidopsis histone deacetylases HD2A, HD2B and HD2C have been implicated in transcriptional repression of genes (Wu et al., 2003). Expression analysis of rice HDAC genes revealed that the expression of several HDACs is influenced by plant hormones as well as abiotic stresses. ABA represses the expression HDT701, HDT702, SRT701 and SRT702 in rice. Abiotic stresses such as cold, manitol and salt also repress SRT701 and SRT702 (Fu et al., 2007). Abiotic stresses induce ABA accumulation, which in turn might repress the HDACs. In Arabidopsis, ABA represses the expression of AtHD2C. Transgenic Arabidopsis plants overexpressing AtHD2C exhibited ABA-insensitive phenotype as well as greater tolerance to salt and drought stresses than the wild type plants. The enhanced tolerance of transgenic plants constitutively overexpressing AtHD2C was partly due to increased expression of ABA-responsive LEA-like genes (RD29B and RAB18), and decreased expression of ABI2, ADH1 (Alcohol Dehydrogenase 1), SKOR (K⁺ outward rectifier), KAT1 and KAT2 (K⁺ inward rectifier) (Sridha and Wu 2006). These results show that ABA mediates abiotic stress responses through dynamic regulation of histone acetylation levels.

Further evidence for roles of histone acetylation in ABA and abiotic stress response was emerged from genetic analysis of hos15-1 (for high expression of osmotically responsive genes) mutant of Arabidopsis. An RD29A::LUC reporter-facilitated genetic screen led to the identification of the mutant hos15-1 mutant, which showed super-induction of RD29A::LUC in response to exogenous ABA, cold and salt stresses. hos15-1 mutants exhibit hypersensitivity to inhibition of germination by ABA and NaCl stress and also hyper-sensitive to freezing stress. HOS15 encodes a protein similar to the human WD-40 repeat protein TBL1 (Transducin Beta-Like protein-1), a component of the chromatin repressor complex involved in histone deacetylation. ABA and abiotic stresses (cold, NaCl) enhanced the expression levels of HOS15. In Arabidopsis, HOS15 interacts with histone H4 and the level of acetylated histone H4 is
higher in *hos15* mutants as compared with that of the wild type. Under cold stress conditions, substantially higher level of acetylated histone H4 is associated with higher expression levels of *RD29A* in the *hos15* mutant than in wild type. Consistent with this, the induction of the *RD29A* gene is higher in the *hos15* mutant than in the wild type under cold stress. Thus, ABA induces expression of *HOS15* which in turn regulates ABA and abiotic stress responses through H4 deacetylation-dependent chromatin remodeling in *Arabidopsis* (Zhu et al., 2007a).

ATP-dependent CRCs also play a role in ABA mediated stress responses. ABA and drought stress induce the expression of *PsSNF5* (*Pisum sativum* *SNF5*), a CRC protein, in germinating embryos as well as vegetative tissues. *PsSNF5* interacts with *Arabidopsis* SWI3-like proteins (SWI3A and SWI3B) in heterologous expression system (Rios et al., 2007). In yeast two-hybrid assay, AtSWI3A and AtSWI3B also interact with FCA, one possible ABA receptor (Sarnowski et al., 2005). Thus, ABA regulates the expression levels of *PsSNF5*, which in turn can mediate chromatin remodeling to regulate ABA-responsive gene expression (Rios et al., 2007).

### 3.3 Regulation of DNA methylation by ABA

DNA methylation is associated with repression of gene expression, while demethylation leads to enhanced expression of genes (Zilberman et al., 2007). Cold stress induced DNA demethylation in maize (Steward et al., 2002). In tobacco, Aluminium, salt and cold stresses induced demethylation of the glycerophosphodiesterase-like protein (*NtGPDL*) gene, followed by upregulation of *NtGPDL* in leaves (Choi and Sano 2007). In tobacco cell-suspension culture, osmotic and salt stresses induced DNA hypermethylation in two heterochromatic loci, which was reversible when cells were returned to non-stress media (Kovarik et al., 1997). Water-deficit stress also induced specific cytosine hypermethylation (CCGG) in the pea genome (Labra et al., 2002). Although, ABA accumulation and role of ABA in gene expression under abiotic stress are well known, the role of ABA in DNA methylation/demethylation dependent gene expression is not understood.

H2B monoubiquitination (K143) regulates the methylation of histones H3 and deubiquitination of H2B directs CpNpG and CpNpN methylation. H2B deubiquitination by the nuclear ubiquitin protease SUP32/UBP26 is required for transcriptional gene
silencing (TGS) (Sridhar et al., 2007). The HUB1 gene has been shown to monoubiquitinate histone H2B, which positively mediate ABA-induced seed dormancy, as bub1 mutation reduced the expression of NCED9 and ABI4, but enhanced the expression of CYP707A2 (Liu et al., 2007). These results suggest a potential role of ABA-mediated processes such as stress response in addition to seed development.

3.4 Small RNAs

Non-coding regulatory small RNAs (~20-24 nt long) namely microRNAs (miRNAs) and small-interfering RNAs (siRNAs) have emerged as important players in plant plant development and stress responses (Jones-Rhoades et al., 2006; Sunkar et al., 2007). RNA-dependent RNA polymerases (RDR) play crucial role in siRNA and miRNA biogenesis. AtRDR6/SGS2/SDE1 (RNA-dependent RNA polymerase6/SUPPRESSOR OF GENE SILENCING2/SILENCING DEFECTIVE1) catalyzes the synthesis of dsRNA from various RNA derived from transgene, viral replication, natural antisense transcripts or miRNA-cleaved mRNA (Vazquez et al., 2004; Borsani et al., 2005; Wassenegger and Krczal 2006). OsRDR6 promoter contains an ABA-responsive DNA cis-element (ACGTG) at the –680 region and exogenous ABA application enhanced the OsRDR6 transcript level in rice cells grown on either acetate or sucrose. Further, ABA silences the isocitrate lyase (ICL) gene (a glyoxylate cycle enzyme) through RDR6 generated siRNA(ICL) mediated post transcriptional gene silencing mechanism (Yang et al., 2008).

Arabidopsis RDR2 is involved in transcriptional gene silencing (TGS) (Chan et al., 2004). Non-coding RNAs can also induce epigenetic changes through chromatin or DNA methylation (Bond and Finnegan 2007). Some of ABA-induced small RNAs and RDRs can play a role in epigenetic regulation of abiotic stress response.

An endogenous siRNA identified from stress-treated Arabidopsis plants is complementary to At2g27152 (AAO3, a drought stress-inducible ABA biosynthesis enzyme). ABA and abiotic stresses have been shown to up-regulate miR402. The predicted target of ABA-upregulated miR402 is At4g34060, which encodes a DNA glycosylase domain protein, DML3 (DEMETER-LIKE PROTEIN 3) similar to ROS1 (Sunkar and Zhu 2004). Two of the homologs of DML3 in Arabidopsis are involved in epigenetic regulation. The DMEDEMETER (DME) DNA glycosylase is required for endosperm gene imprinting and seed viability (Choi et al., 2002). The ROS1 DNA
demethylase has a key role in the plasticity of the epigenome (Gong et al., 2002; Agius et al., 2006; Zhu et al., 2007b). Probably, ABA-induced RDR6 might synthesize siRNA that target AAO3 and thus regulate the cellular levels of ABA under stress conditions. ABA-induced miR402 might reduce the transcript levels of DML3, which in turn can result in increase in methylation levels at certain loci and thus repression of gene expression.

4. Abiotic Stress Memory
Abiotic stresses induce changes in the genome is necessary for stress memory. UV-C radiation or flagellin (an elicitor of plant defense) stress-induced changes in the genome has been shown to be dominant trait and transmitted through both the maternal and the paternal crossing partner in *Arabidopsis* (Molinier et al., 2006). Progeny of tobacco mosaic virus (TMV) infected plants showed enhanced frequency of the somatic and meiotic recombination rates at certain loci due to DNA hypomethylation. Thus stress memory appears to be inherited through epigenetic changes (Boyko et al., 2007). ABA accumulation in vegetative tissues is induced by abiotic stresses. ABA also enhances the somatic recombination frequency (Gong et al., unpublished data). As discussed in this review, ABA induces chromatin remodeling. Chromatin remodeling regulates transcription, recombination, replication and genome organization (Meyer 2001). Probably ABA might also regulate DNA methylation through DML3 and siRNAs. Under abiotic stresses ABA mediates reduction in plant growth. One of the mechanisms of abiotic stress induced growth arrest under abiotic stress is regulated by chromatin remodeling (Mlynárová et al., 2007). Abiotic stress-induced inheritable epigenetic state might have an adaptive advantage but also a negative impact yield of crops, as stress memory can inhibit the expression of full potential of crops. Hence it is imperative to understand the role of ABA in abiotic stress memory to utilize them for agricultural advantage.

Concluding Remarks and Perspectives
ABA regulates plant development and abiotic stress tolerance through genetic and epigenetic mechanisms. Role of ABA in epigenetic processes is just beginning to emerge (Figure 1). Seed development and germination are vital processes not only for plant survival but also for other organisms including humans, as successful agriculture depends
upon these processes. ABA plays pivotal role in seed development and dormancy by
direct induction of gene expression or histone ubiquitination, acetylation and methylation
dependent chromatin remodeling. In turn, these epigenetic processes might regulate ABA
levels through change in expression of genes involved in ABA metabolism.
Transpiration, stomatal opening and plant growth are regulated under abiotic stresses
through repression of histone deacetylation and chromatin modeling. ABA-regulated
histone acetylation levels either through ABA-repression of histone deacetylases or
repression of $\text{HOS15}$ (a protein similar to the human WD-40 repeat protein TBL1) plays
a key role in chromatin remodeling, abiotic stress responsive gene expression and stress
tolerance of plants (Figure 2). ABA also probably regulates siRNA pathways and DNA
methylation under abiotic stresses. It is not known whether ABA-induced epigenetic
changes in response to stresses are heritable through mitosis as well as meiosis and their
adaptive advantage. Further studies on ABA-regulated epigenome will be necessary for
better understanding of seed development, storage reserve accumulation in seeds, seed
dormancy, plant development, abiotic resistance and engineering of these processes for
agricultural use.

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References


Figure Legends

Fig. 1 ABA-mediated epigenetic processes regulate seed maturation, dormancy and germination inhibition. *HISTONE MONOUBIQUITINATION1 (HUB1)* monoubiquitinates H2B, which in turn regulates ABA-levels by inducing NCED and repressing *CYP707A2*. Enhanced ABA level induces ABI4 expression and H2B monoubiquitination induces the expression of *DOG1*, *ATS2* and *PER1*. These proteins in turn induce seed dormancy. VP1/ABI3-like (VAL proteins recruits SWI/SNF class chromatin remodeling complex, PIKLE (PKL). PKL and HDACs dependent chromatin remodeling repress the expression of genes involved in seed maturation and stress induced germination arrest. ABA probably down regulates HDACs (HDA6, HDA19) and also induce the expression of these genes involved in seed maturation and stress induced germination arrest (ABA2, Short chain dehydrogenase; ABI, ABA Insensitive; AAO3, Abscisic aldehyde oxidase; ATS2, a caleosin-like protein; CYP707A, ABA 8’ hydroxylase; DOG1, DELAY OF GERMINATION 1; FUS3, FUSCA3; H2B, Histone 2B; HDACs, histone deacetylases; LEC1, LEAFY COTYLEDON 1; NCED, 9 cis-epoxycarotenoid dioxygenase; PER1, peroxiredoxin family of antioxidants; UQ, ubiquitin; ZEP, Zeaxanthin epoxidase).

Fig. 2 ABA-mediated chromatin remodeling regulates stress responsive gene expression and stress tolerance. Abiotic stresses induce accumulation of ABA. ABA enhances the expression levels of *HOS15*, which encodes a protein similar to the human WD-40 repeat protein TBL1 (*Transducin Beta-Like protein-1*), a component of the chromatin repression complex involved in histone deacetylation. Conversely, ABA can also inhibit histone deacetylation through repression of HDACs. ABA and drought induce an ATP-dependent chromatin remodeling complex protein, SNF5. Histone deacetylation dependent and ATP-dependent chromatin remodeling complexes regulate gene expression and stress response (Ac, acetyl group; H4, Histone 4; HDACs, histone deacetylases; HOS15, High expression of Osmotically responsive genes 15)
Abiotic Stresses

ABA

HDACs  HOS15  SNF5

AcH4  Ac  H4

Chromatin Remodeling

Gene Expression/Repression & Stress Responses