Function and regulation of Arabidopsis histone deacetylases in stress response

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ABBREVIATIONS

ABA: Abscisic Acid

ABI3: Abscisic Acid Insensitive 3

AGL19: Agamous-like 19

CAB2: Chlorophyll A/B-binding Protein 2

ChIP: Chromatin Immunoprecipitation

CK: Cytokinins

Col-0: Columbia-0

Cys: Cysteine

DAB: 3,3′-diaminobenzidine

ERF1: Ethylene Response Factor1

FT: Flowering Locus T

FUS3: Fusca 3

GA: Gibberellins

GNAT: GCN5-related N-terminal Acetyltransferases

GSH: Glutathione

GSNO: S-nitrosoglutathione

HAF: TATA Binding Protein-associated Factor Family

HAT-A: Type A HATs

HAT-B: Type B HATs

HATs: Histone Acetyltransferases

HDACs: Histone Deacetylases

H_2O_2: Hydrogen Peroxide

HSFA: Heat Stress Transcription Factor

HSL1: High-level Expression of Sugar-inducible Gene 2-like 1

HY5: Elongated Hypocotyl 5

IAA19: Indole-3-acetic Acid Inducible 19

IC: Isochorismate

ICS: Isochorismatesynthase

INA: 2,6-dichloro-isonicotinic Acid

IPL: Isochorismate Pyruvate Lyase

JA: Jasmonic Acid

MBF1c: Multiprotein Bridging Factor 1c

MYST: MOZ, Ybf2/Sas3, Sas2 and Tip60

NAD: Nicotinamide Adenine Dinucleotide

NES: Nuclear Export Signal

NLS: Nuclear Localization Signal

NPR1: Non-expressor of PR Genes1

NO: Nitric Oxide

$^{\bullet}OH$: Hydroxyl Radical

$O2^{\bullet-}$: Superoxide

$^{1}O_2$: Singlet Oxygen

PAL: Phenylalanine Ammonia-lyase

PCR: Polymerase Chain Reaction

PEG: Polyethylene Glycol

PHY: Phytochromes

PIF4: Phytochrome Interacting Factor 4

PKL: Pickle

PP2C-6-6: Phosphatase 2C

Prx: Peroxiredoxin

PR1: Pathogenesis-related Gene 1

p300/CBP: CREB-binding Protein

RBCS-1A: Ribulose Bisphosphate Carboxylase Small Chain 1A

RNS: Reactive Nitrogen Species

ROS: Reactive Oxygen Species

RPD3: Reduced Potassium Deficiency 3

RT-qPCR: Reverse Transcription-quantitative PCR

SA: Salicylic Acid

SAG: SA β-glucoside

SAR: Systemic Acquired Resistance

SCL15: Scarecrow-like15

SIR2: Silent Information Regulator 2

SNO: S-nitrosylation

SOH: S-sulphenation

SO_2H: S-sulphination

SO_3H: S-sulfonation

TAFII250: TATA Binding Protein-associated Factor

TPL: Topless

Trx: Thioredoxin

TSA: Trichostatin A

UVH6: UV-hypersensitive 6

Ws: Wassilevskija

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INTRODUCTION

1.1 Epigenetic contribution to regulation of plant development and stress response

1.1.1 Definition of epigenetics

The term epigenetics was coined by Conrad H. Waddington to understand the mechanisms by which genes bring about phenotypic effects in 1942 (Heard and Martienssen, 2014). With the development of molecular genetics, epigenetics now refers to "the study of mitotically and/or meiotically heritable changes in gene function that cannot be explained by changes in DNA sequence" (Grossniklaus et al., 2013). Presently, epigenetic research mostly focuses on the study of chromatin modification mechanisms that affect gene expression states and their maintenance or resetting during mitosis and/or meiosis, which are independent of changes in the DNA sequence (Quadrana and Colot, 2016).

Over the last few decades, studying the mechanisms of epigenetic regulation in plants has gained great interest, not only as a subject of basic biological research but also as a new source of beneficial trait development for plant breeding (Gallusci et al., 2017). Epigenomic study and functional characterization of plant epigenetic regulators have revealed that epigenetic modifications play important roles in a diverse range of developmental processes and stress-responsive pathways **(Fig 1)**. Therefore, deciphering precise epigenetic mechanisms regulating important developmental and physiological processes and characterizing phenotypic consequences caused by epigenetic changes should provide novel strategies to improve plant adaptation to environmental challenges and to increase plant productivity.

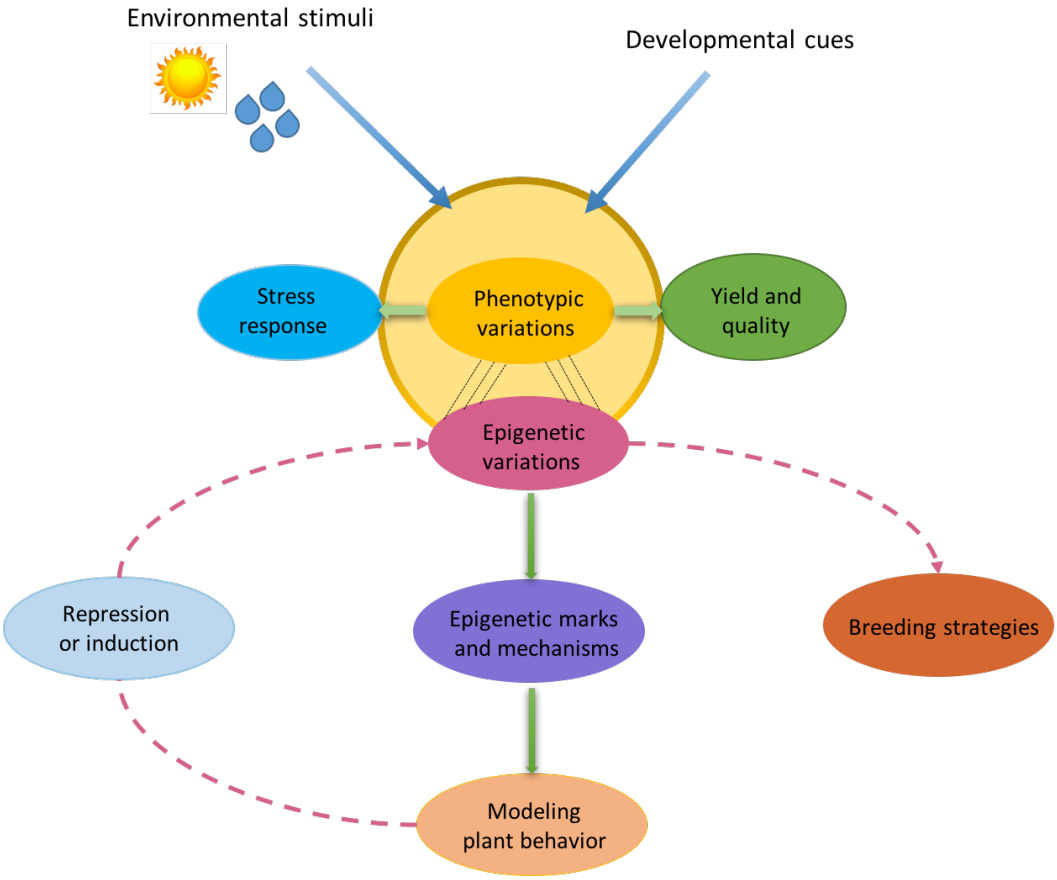

Figure 1. The model of epigenetic modifications in developmental processes and stress-responsive pathways. Phenotypic variations that rely on epigenetic regulation are key levers for improving plant adaptation to stresses, yield, and quality. Environmental stimuli and/or developmental cues are considered to be key factors to induce epigenetic variations. Thus, understanding mechanisms of epigenetic variations is essential to develop model-driven breeding approaches (adapted from Gallusci et al., 2017).

1.1.2 Histone modification

1.1.2.1 The structural chromatin organization

In eukaryotes, genomic DNA is packaged in a highly organized chromatin structure to fit inside the physically restricted space of the nucleus (Richmond and Davey, 2003). The basic element of chromatin is nucleosome, which is formed by approximately 147 base pairs of DNA wrapped around an octamer of histone proteins H2A, H2B, H3, and H4 **(Fig 2)** (Joti et al., 2012). The overall structure of chromatin displays dynamic changes. Relaxed chromatin structure allows RNA and DNA polymerases accessing to genomic DNA for gene transcription and DNA replication. Epigenetic modification of chromatin affects the accessibility and effectiveness of transcription and replication machineries. Epigenetic modifications of chromatin include DNA methylation, covalent posttranslational modifications of histones (Kouzarides, 2007; Pfluger and Wagner, 2007; Zhang et al., 2007), ATP-dependent chromatin remodeling (de la Serna et al., 2006; Jerzmanowski, 2007), and replacement of canonical histones with specific histone variants (March-Diaz and Reyes, 2009; Zlatanova and Thakar, 2008). Among these chromatin modifications, histone post-translational modifications have been shown to play important roles in gene expression, especially in regulation of developmental and stress-responsive genes in plants. Histone modifications include acetylation, methylation, and ubiquitination of lysine residues, in addition to many others. I will focus on plant histone acetylation/deacetylation dynamics and recent advances in histone acetylation function in regulation of plant developmental and stress-responsive genes.

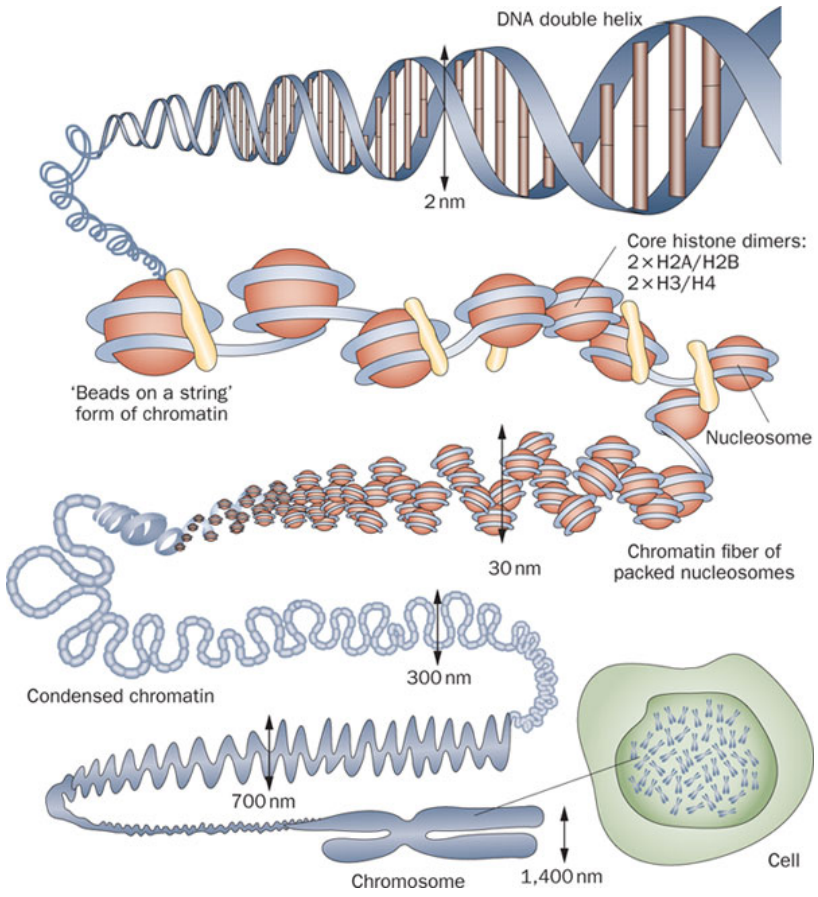

Figure 2. Organizational network of chromatin in the cell. Genomic DNA is complexed with histones and nuclear proteins to form chromatin, of which nucleosome is the basic structure. The core particle of nucleosome is composed of 147 bp of genomic DNA wrapped around a histone octamer that consists of two copies of each histone (H2A, H2B, H3 and H4). Condensation of nucleosomes and chromatin fibers forms higher order chromatin structures (Tonna et al., 2010).

1.1.2.2 Histone acetylation/deacetylation

In plant, many physiological processes such as development and stress response are known to be regulated by histone modification. Histone modifications such as acetylation, methylation, and ubiquitination of lysine residues mainly occur at N-termini of the proteins (called histone tails) **(Fig 3)** (Berger, 2007; Fuchs et al., 2006; Zhang et al., 2007a). A same lysine residue can be either methylated or

acetylated (Roudier et al., 2011). In addition, histone acetylation, as well as methylation, is reversible, which therefore provides flexibility of gene regulation allowing plant rapid response to developmental and environmental clues.

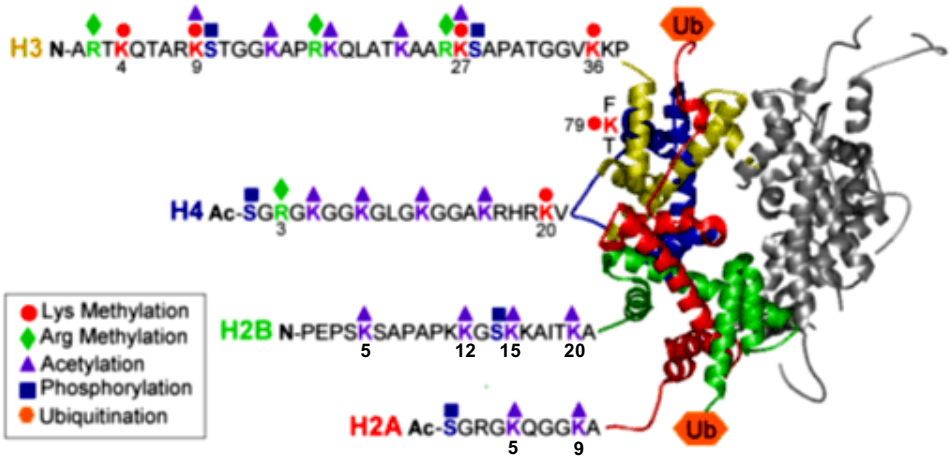

Figure 3. Major kinds of histone N-terminal modifications. The numbers below the amino acids correspond to the position of the modified residues at the N-terminus of the histone proteins.

Histone lysine acetylation is considered as a major factor that facilitates chromatin relaxation, and hence gene activation (Kouzarides, 2007; Kuo and Allis, 1998). In generally, strong acetylation of histones relaxes chromatin structure, allowing access of transcriptional initiation complex or transcription factors and RNA polymerase to genomic DNA. Weak or no histone acetylation leads to chromatin compaction and gene repression (Waterborg, 2011). The histone acetylation/deacetylation dynamics is controlled by histone acetyltransferases (HATs) and histone deacetylases (HDACs) **(Fig 4)** (Kuo and Allis, 1998; Brownell and Allis, 1996). HATs transfer acetyl moiety from acetyl-CoA to ε-amino group of lysine residues. In contrast, HDACs catalyze the removal of acetyl groups from acetylated lysine residues of histones. Histone H3 (at K4, K9, K14, K18, K23, K27, and K36) and H4 (at K5, K8, K12, K16, and K20) are found to be acetylation/deacetylation targets in *Arabidopsis* and other plants, as well as in yeast and animals (Shahbazian and Grunstein, 2007).

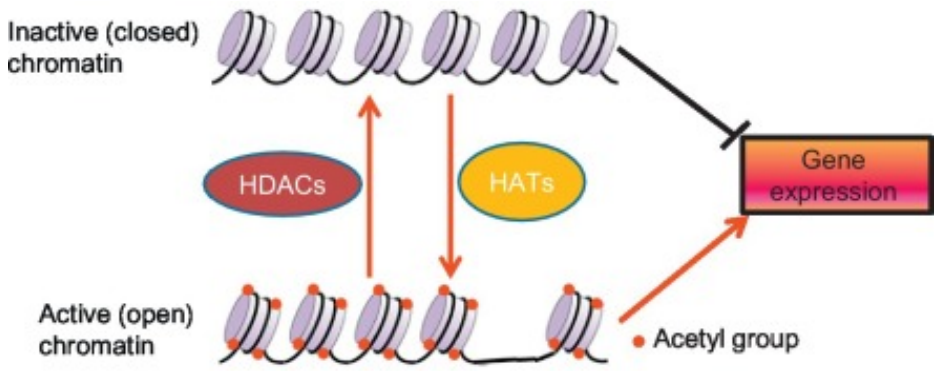

Figure 4. Histone acetylation affects chromatin condensation and gene expression. Histone lysine acetylation (red points) is controlled by HATs and HDACs. Lysine acetylation weakens DNA-histone contacts and inhibits condensation of nucleosomes, thereby allowing access of transcription factors and transcriptional machinery to DNA (Liu et al., 2016).

1.1.3 Histone acetyltransferases

HATs activities were first characterized at the early 1970's. Two types (type A and B) of HATs were subsequently identified according to their substrate specificity and intracellular localization (Roth et al., 2001). Type B HATs (HAT-B) are cytoplasmic proteins that catalyze newly synthesized histone acetylation in cytoplasm, particularly at H4K5 and H4K12 sites (Verreault et al., 1998). Although HAT-B are conserved in structure and function from fungi, plants to mammalian cells, the overall sequence homology is rather low (Kolle et al., 1988; Parthun, 2007). A HAT-B (U90274) was purified and characterized from maize (Eberharter et al., 1996). Maize HAT-B is composed of two subunits, p50 and p45. The 50 kDa subunit has the catalytic function of acetylation and is homologous to yeast Hat1p, and the 45 kDa subunit is associated to numerous proteins that bind core histones (Lusser et al., 1999). In addition, maize HAT-B is mostly abundant in the cytoplasm, but a significant proportion of p50 subunit accumulates in nuclei, suggesting that this HAT-B has additional nuclear functions (Lusser et al., 1999). A homolog of HAT-B in *Arabidopsis* (AtHAG2, At5g56740) was predicted by sequence analysis (Pandey et al., 2002), but the function of AtHAG2 remains unclear. The type A HATs (HAT-A) are

localized in the nucleus and are responsible for acetylation of nucleosomal core histones. Hence, they are directly related to regulation of chromatin structure and gene transcription (Brownell and Allis, 1996).

Based on sequence similarity, the plant HATs are grouped into four categories: (1) the GNAT (GCN5-related N-terminal acetyltransferases) superfamily, (2) the MOZ, Ybf2/Sas3, Sas2 and Tip60 (MYST) superfamily, (3) the CREB-binding protein (p300/CBP) family, (4) the TATA binding protein-associated factor ($TAF_{II}250$) family (HAF) (Carrozza et al., 2003). A total of 12 *Arabidopsis* HATs genes have been identified **(Table 1)**, of which three belong to the GNAT superfamily (*HAG1/AtGCN5*, *HAG3/AtELP3*, and *HAG2*), two to the MYST superfamily (*HAG4/HAM1* and *HAG5/HAM2*), five to the p300/CBP family (*HAC1*, *HAC2*, *HAC4*, *HAC5*, and *HAC12*), and two to the $TAF_{II}250$ family (*HAF1* and *HAF2*) (Pandey et al., 2002).

Table 1 HATs protein genes characterized in *Arabidopsis*

GENE name	Accession	Function	References
GNAT family			
atGCN5/HAG1/HAT1	At3g54610	Root and shoot development Seeds fatty acid composition miRNA production Flower development Light signaling and hypocotyls growth Low temperature response Ethylene responses Heat response	Benhamed et al., 2006; Hu et al., 2016; Kim et al., 2009; Kornet and Scheres, 2005; Kornet and Scheres, 2009; Long et al., 2006; Poulios and Vlachonasios, 2016; Wang et al., 2016; Vlachonasios et al., 2003;
HAG2	At5g56740	Unknown	Unknown
HAG3/atELP3/atELO3	At5g50320	Cell proliferation UV-B responses	Nelissen et al., 2005 Fina and Casati, 2015
MYST family			
HAM1	At5g64610	Flowering time regulation and gamete	Latrasse et al., 2008; Xiao et al., 2013
HAM2	At5g09740	Flowering time regulation and gamete	Latrasse et al., 2008; Xiao et al., 2013
CBP/P300 family			
HAC1	At1g79000	Sugar response Flowering time regulation Ethylene signaling pathway UV-B responses	Deng et al., 2007; Heisel et al., 2013; Julieta et al., 2017; Li et al., 2014
HAC2	At1g67220	Unknown	Unknown
HAC4	At1g55970	Pleiotropic developmental processes	Li et al., 2014
HAC5	At3g12980	Ethylene signaling pathway Pleiotropic developmental processes	Li et al., 2014 Li et al., 2014
HAC12	At1g16710	Pleiotropic developmental processes	Li et al., 2014
TAFII250			
HAF1	At1g32750	UV-B responses	Julieta et al., 2017
HAF2/TAF1	At3g19040	Light signaling and hypocotyls growth Resistance to genotoxic stress and viability of the male gametophyte	Bertrand et al., 2005; Benhamed et al., 2006; Waterworth et al., 2015

1.1.3.1 Functions of histone acetyltransferase AtGCN5

Analysis of T-DNA insertion mutants or RNAi plants has revealed that HATs play diverse and important roles in many aspects of plant developmental and physiological processes. In particular, HAG1/AtGCN5 (At3g54610) has been shown to be essential for histone acetylation, gene expression, and plant development and is considered as the major HAT in plants (Servet et al., 2010). HAG1/AtGCN5 shows a HAT activity *in vitro* that specifically acetylates histone H3K14 (Bertrand et al., 2003; Earley et al., 2007). AtGCN5 appears to be a phosphorylated protein (Servet et al., 2008). Servet et al. found that a phosphatase 2C protein (PP2C-6-6) interacts with AtGCN5 *in vivo* and that phosphorylated AtGCN5 can be dephosphorylated by PP2C-6-6 *in vitro*. In addition, *pp2c-6-6* mutations induced H3K14 hyperacetylation *in vivo* (Servet et al., 2008), suggesting that AtPP2C-6-6 may modulate the HAT activity of AtGCN5 by posttranslational modification.

AtGCN5 mutants (*gcn5-1*, *gcn5-2*, *hag1-5*, and *hag1-6*) showed various abnormal phenotypes. The defects included dwarfism, loss of apical dominance, aberrant meristem function, root and leaf development as well as short petals and stamens, and floral organ identity (Bertrand et al., 2003; Kornet and Scheres, 2009; Long et al., 2006; Vlachonasios, 2003), suggesting that AtGCN5 is a versatile regulator of plant development. AtGCN5 also plays essential roles in regulating gene expression in plant responses to environmental conditions (Servet et al., 2010). In plants, light signals received by distinct wave-length-specific photoreceptors, such as phytochromes (phy) and cryptochromes, cause a series of cascade reactions to activate the downstream transcription regulators, then rapidly inducing the expression of a large number of genes (Jiao et al., 2007). The *gcn5-1* mutant showed a light-hyposensitive phenotype (long hypocotyl) under far-red light condition. The transcripts of light-inducible genes, such as chlorophyll A/B-binding protein 2 (*CAB2*) and ribulose bisphosphate carboxylase small chain 1A (*RBCS-1A*), were seriously reduced in the *gcn5-1* mutants (Benhamed et al., 2006). Moreover, chromatin immunoprecipitation (ChIP) assays showed that AtGCN5 binds directly to the core promoter regions of these two light-responsive genes (Benhamed et al., 2006). These data indicated that AtGCN5 is a positive regulator of a subset of light-inducible genes. In addition, genetic analysis showed that *AtGCN5* is epistatic to *ELONGATED*

HYPOCOTYL5 (*HY5*) that is required for responding to light signals, and that HY5 and AtGCN5 share many genomic targets, especially early light-responsive genes (Benhamed et al., 2006; Benhamed et al., 2008), indicating that AtGCN5 and HY5 are involved in the same regulatory pathway in controlling light-responsive genes. Another Arabidopsis HAT gene, *HAF2*/*TAF1* (At3g19040), was also shown to be involved in regulating histone acetylation and transcription of light-responsive genes (Bertrand et al., 2005). However, RNA gel blot analysis of *gcn5*, *taf1* and *gcn5 taf1* double mutants indicated that the two HATs regulate only a subset of light-inducible genes expression and they have distinct light-responsive target genes, which is in agreement with the different photomorphogenic phenotypes observed in the respective mutants (Benhamed et al., 2006; Bertrand et al., 2005). Moreover, *taf1 hy5* double mutations produced a synergistic effect on enhancing hypocotyl elongation, whereas the hypocotyl lengths of *gcn5 hy5* were similar to those of the *gcn5* single mutants (Bertrand et al., 2005). Therefore, AtGCN5 and TAF1 likely regulate light-responsive genes through different signaling pathways **(Fig 5)**.

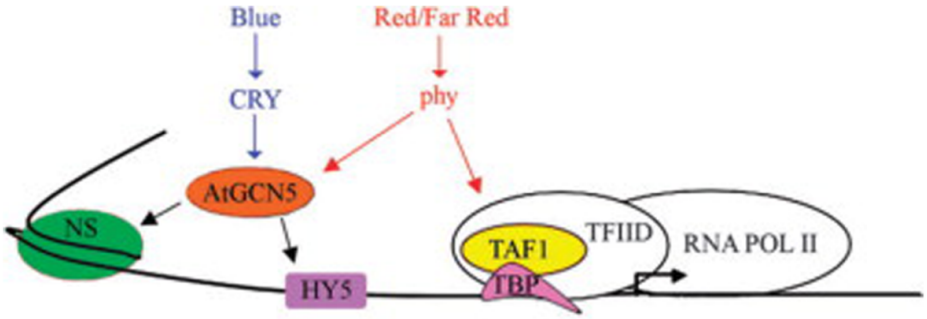

Figure 5. Model of Integration of Light Signaling by Histone Acetylation Enzymes. AtGCN5 responds to both blue and red/far-red light signals received by cryptochromes (CRY) and phytochromes (phy), respectively, to induce histone acetylation, while TAF1 integrates red/far-red light signals for histone acetylation and gene activation. AtGCN5 can be recruited to the promoter by interacting with DNA-binding transcription factors such as HY5 and/or with acetylated histone lysine residues of nearby nucleosomes (NS), while TAF1 is supposed to be recruited by interacting with the TATA-binding protein (TBP) within the TFIID complex to support RNA polymerase II (POL) transcription (adapted from Servet et al., 2010).

AtGCN5 was also found to be associated to activation of heat stress-responsive genes and thermotolerance (Hu et al., 2015). Loss of function of *AtGCN5* considerably affected the expression of heat stress transcription factor2 (*HSFA2*) and 3 (*HSFA3*), as well as transcription factors *Multiprotein Bridging Factor1c* (*MBF1c*) and *UV-HYPERSENSITIVE 6* (*UVH6*) that are required for plant thermotolerance, leading to a defective phenotype in *gcn5* mutants after heat treatment (Hu et al., 2015). H3K9 and H3K14 acetylation levels at the *UVH6* promoter regions were significantly enhanced in the wild type under heat stress condition, whereas *gcn5* mutants showed significantly decreased H3K9 and H3K14 acetylation at promoters of *UVH6* under heat-treatment. Constitutive expression of *UVH6* in the *gcn5* background partially restored the normal thermotolerant phenotype, suggesting a direct role of AtGCN5 in regulation of *UVH6* expression (Hu et al., 2015).

1.1.4 Histone deacetylases

Sequence analysis revealed three families of HDACs in plants (Pandey et al., 2002). The first family is named RPD3/HDA1 superfamily, which is homologous to the Reduced Potassium Deficiency 3 (RPD3) in yeast and animal cells (Hollender and Liu, 2008). The second is Silent Information Regulator 2 (SIR2) family. SIR2 HDACs are structurally-distinct from other HDACs and their deacetylation activities depend on nicotinamide adenine dinucleotide (NAD^+) (Frye, 2000). The third family, the so-called HD2 family, is initially characterized in maize (Lusser et al., 1997) and appears to be present only in plants (Wu et al., 2000). In *Arabidopsis*, a total of 16 HDACs genes have been identified **(Table 2)**, of which 10 belong to the RPD3/HDA1 superfamily that can be subdivided into 3 classes according to the sequences similarity: Class I (*HDA1/HDA19/HD1*, *HDA6/AXE1*, *HDA7* and *HDA9*), Class II (*HDA5*, *HDA15* and *HDA18*) and Class III (*HDA2*). HDA8 and HDA14 are unclassified proteins though they share the conserved amino positions of Class II proteins. In addition, HDA10 (At3g44660) and HDA17 (At3g44490) possess an incomplete HDAC domain. In *Arabidopsis*, the SIR2 family contains two members (*SRT1* and *SRT2*) and the HD2 family has four (*HDT1/AtHD2A*, *HDT2/AtHD2B*, *HDT3/AtHD2C,* and *HDT4/AtHD2D*).

Table 2 Summary of HDACs genes characterized in *Arabidopsis*

GENE name	Accession	Function	References
RPD3-HDA1 family			
HDA1/HDA19/HD1	At4g38130	Seed maturation and dormancy Flower development Ethylene, JA, SA pathway and basal defense Light signaling and hypocotyls growth High temperature response Leaf morphogenesis Root cell elongation	Benhamed et al., 2006; Chen et al., 2015; Choi et al., 2012; Gonzalez et al., 2007; Kim et ai., 2008; Long et al., 2006; Perrella et al., 2013; Tanaka et al., 2008; Tian et al., 2003, 2005; Tian and Chen, 2001; Wang et al., 2013b; Zhou et al., 2005; Zhou et al., 2013;
HDA2	At5g26040	Unknown	Unknown
HDA5	At5g61060	Flowering time	Luo et al., 2015
HDA6/AXE1	At5g63110	Seed development and germination ABA and salt stress response Leaf morphogenesis Circadian regulation Flowering controlling JA signaling Cold stress response and ethylene pathway Light signaling DNA methylation Brassinosteroid signaling	Aufsatz et al., 2002; Gu et al., 2011; Hao et al., 2016; Luo et al., 2012a, 2012b; Perrella et al., 2013; Scofield and Murray, 2006; Tanaka et al., 2008; Tessadori et al., 2009; Thines et al., 2007; To et al., 2011; Wang et al., 2013a; Wu et al., 2008; Yu et al., 2011; Zhu et al., 2011;
HDA7	At5g35600	Seed germination, plant growth, female gametophyte development and embryogenesis	Aiese-Cigliano et al., 2013a
HDA8	At1g08460	Unknown	Unknown
HDA9	At3g44680	Flowering time Salt stress response Seed development Leaf senescence	Chen et al., 2016; Kang et al., 2015; Kim et al., 2013; Kim et al., 2016;

			Zanten et al., 2014; Zheng et al., 2016;
HDA10	At3g44660	Unknown	Unknown
HDA14	At4g33470	Deacetylate a-tubin	Tran et al., 2012
HDA15	At3g18520	Phytochrome A/B pathway and hypocotyls growth Negative component of PHTB-dependent seed germination	Liu et al., 2013b Gu et al., 2017
HDA17	At3g44490	Male gametophyte	Li et al., 2014
HDA18	At5g61070	Root hair development	Liu et al., 2013
SIR2 family			
SRT1	At5g55760	Cell dedifferentiation	Lee et al., 2016
SRT2	At5g09230	Basal defense of pathogen Mitochondria energy metabolism	Wang et al., 2010 Koing et al., 2014
HD2 family			
AtHD2A/HDT1/HDA3	At3g44750	Leaf morphogenesis	Kidner and Martienssen, 2004; Ueno et al., 2007
AtHD2B/HDT2/HDA4	At5g22650	Leaf morphogenesis	Kidner and Martienssen, 2004; Ueno et al., 2007
AtHD2C/HDT3	At5g03740	ABA pathway, salt stress and seed germination	Colville et al., 2011; Luo et al., 2012a
AtHD2D/HDT4/HDA13	At2g27840	Plant growth, development and abiotic stresses	Han et al., 2016

1.1.4.1 The SIR2 family

Arabidopsis SIR2 members have been shown to be involved in cellular dedifferentiation and response to pathogens (Lee et al., 2016; Wang et al., 2010). *AtSRT1* transcript has been shown to be significantly up-regulated in leaf explant-derived calli, whereas the expression of *AtSRT2* is highly repressed (Lee et al., 2016), suggesting distinct functions between *AtSRT1* and *AtSRT2* in cellular dedifferentiation. The expression of *AtSRT2* was also down-regulated in response to pathogen *Pseudomonas syringae pv. tomato* DC3000 infection in a salicylic acid (SA)-independent manner (Wang et al., 2010). The mutation of *AtSRT2* produced an enhanced pathogen-resistance phenotype and increased expression of pathogenesis-related gene 1 (*PR1*). The *AtSRT2* mutants also showed increased expression of *PAD4*, *EDS5* and *SID2* that are essential for SA biosynthesis (Wang et al., 2010), suggesting that AtSRT2 may regulate plant basal defense by suppressing SA biosynthesis. Interestingly, AtSRT2 has been recently shown to locate predominantly in the inner mitochondrial membrane and deacetylate a small subset of protein complexes mainly associated with energy metabolism and metabolite transport (Konig et al., 2014), implying a novel function of SIR2 proteins in energy metabolism as metabolic protein Lys deacetylases.

A rice (*Oryza sativa*) SIR2 homolog, *OsSRT1*, has been shown to have a function to safeguard against genome instability and cell damage by regulating histone H3K9 acetylation (Zhong et al., 2013). Overexpression of *OsSRT1* in transgenic rice led to increased tolerance to oxidative stress, whereas down-regulation of *OsSRT1* resulted in H_2O_2 production, genomic DNA fragmentation, cell death, and lesions mimicking plant hypersensitive responses during incompatible interactions (Huang et al., 2007). Transcriptome analysis showed that many genes involved in hypersensitive response, programmed cell death, stress response, and metabolism are up- or down-regulated in RNA interference lines of *OsSRT1* (*OsSRT1 RNAi*) in rice, suggesting a general role of SRT1 in gene expression. In addition, the transcription of many transposons and retrotransposons was also activated in *OsSRT1 RNAi* plants and ChIP-seq analysis of the genomic binding sites of OsSRT1 and H3K9ac in *OsSRT1 RNAi* and wild type plants further revealed that many of them are direct targets of OsSRT1 for H3K9 deacetylation (Huang et al., 2007; Zhong et al., 2013), suggesting that OsSRT1 is

directly involved in transposable element repression.

1.1.4.2 The HD2-type HDACs family

The HD2 family is a group of plant-specific histone deacetylases that are highly conserved in plants (Grandperret et al., 2014). Most of these HD2 members have a conserved N-terminal domain, a central acidic domain, and variant C-terminal domain **(Fig 6)** (Pandey et al., 2002). The conserved N-terminus contains the (M) EFWG motif in the N-terminal end followed by the HDAC catalytic domain of approximately 100 amino acids (Dangl et al., 2001). A large central acidic domain mainly comprises Asp residues (Bourque et al., 2011; Brosch et al., 1996). Several Ser and/or Thr residues in these acidic domains are predicted to be phosphorylated by casein kinase 2α that is involved in many fundamental biological processes (Litchfield, 2003). In addition, all HD2 proteins contain a functional nuclear localization signal (NLS) motif and some of them also possess a nuclear export signal (NES) in the C-terminal domain (Sridha and Wu, 2006; Zhou et al., 2004), suggesting that HD2 HDACs may shuttle from the nucleus to the cytosol under varying environmental conditions. Some of HD2 members also contain a C2H2-type Zn^{2+} finger domain in the C-terminal part, the function of which is still unclear. It is proposed that this domain might be also involved in export of HDACs from the nucleus to the cytosol (Grandperret et al., 2014).

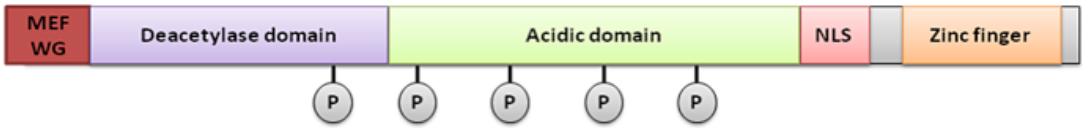

Figure 6. Schematic representation of plant conserved type-II histone deacetylases (HD2) domains. This schematic structure of plant HD2s is based on AtHDT1. MEFWG, conserved pentapeptide in the N-terminal end; NLS, nuclear localization signal; P, putative casein kinase 2α-specific phosphorylation site (Grandperret et al., 2014).

Of the *Arabidopsis* HD2 family, *AtHD2A*, *AtHD2B*, and *AtHD2C* were found to be highly expressed in ovules, embryos, shoot apical meristems as well as primary leaves and during somatic embryogenesis (Zhou et al., 2004), suggesting that AtHD2s might be essential for embryo development. The highly overlapping expression of these three AtHD2s also implies a possibility of functional redundancy. *AtHD2D* has distinct spatial expression patterns from the other three members, in that its transcript accumulates in stems, flowers, and young siliques (Zhou et al., 2004), suggesting a diverged function for AtHD2D. It has been shown that AtHD2A, AtHD2B and AtHD2C localize in the nucleus and play roles in fundamental physiologic processes such as seed germination and stress response (Colville et al., 2011; Luo et al., 2012; Yano et al., 2013). Both downregulation and overexpression of *AtHD2A* resulted in abortive seed development. The overexpression of *AtHD2A* also induced the repression of genes that are involved in seed development and maturation (Colville et al., 2011). Moreover, AtHD2A and AtHD2C were shown to be antagonistic regulators on seed germination: *athd2a* mutants showed higher germination rates compared to wild type, whereas the seed germination was inhibited in the *athd2c* mutant lines. The *athd2a athd2c* double mutations restored the normal germination rate (Colville et al., 2011). In addition, AtHD2B was also found to play a role in seed dormancy and germination (Yano et al., 2013). Collectively, the data suggest specific functions of HD2 family members in seed development.

1.1.4.3 The RPD3/HDA1 superfamily

1.1.4.3.1 Functions of RPD3/HDA1 family HDACs in plant development

The involvement of RPD3/HDA1 family HDACs in plant development has been reported. Among the *Arabidopsis* RPD3 members, two of Class I HDACs, *AtHDA19* and *AtHDA6*, are well characterized and are shown to exhibit divergent and overlapping functions in a range of plant development processes (Hollender and Liu, 2008). Western blots showed that *athda19* mutation induces hyperacetylation of histone H3K9 and H4K12 as well as H4 lysine residues (Tian and Chen, 2001; Tian et al., 2003; Tian et al., 2005), whereas over-expression of *AtHDA19* reduced acetylation of histone H3 lysine residues (Zhou et al., 2005), suggesting a global

HDAC activity of AtHDA19. AtHDA6 also has a general deacetylase activity, as it removes the acetyl group from H3K9ac, H3K14ac, H3K18ac, H3K23ac, H3K27ac, H4K5ac, H4K8ac, and H4K12ac (Hollender and Liu, 2008). Recently, a novel H3 lysine acetylation, H3K36ac, was identified in *Arabidopsis*. AtHDA19, but not AtHDA6, is required for removal of H3K36ac from target genes *in vivo* (Mahrez et al., 2016). It has been shown that HDA6 is also involved in DNA methylation and regulation of rRNA genes, in addition to histone acetylation and gene expression (Aufsatz et al., 2002; Earley et al., 2006; Probst et al., 2004). There is evidence indicating that HDA6 and HDA19 play redundant roles in repressing embryonic properties after germination via regulation of embryo-specific gene expression in *Arabidopsis* (Tanaka et al., 2008). HDA6 RNA-interference (HDA6-RNAi) lines did not show any abnormal growth after germination, but displayed an arrested post-germination growth phenotype following treatment with a low concentration of trichostatin A (TSA, an HDAC inhibitor). Compared with wild-type plants, the expression of embryo-specific transcription factors, such as *FUSCA3* (*FUS3*) and *ABSCISIC ACID INSENSITIVE3* (*ABI3*), was strongly increased in HDA6-RNAi plants upon TSA treatment (Tanaka et al., 2008). Similarly, untreated *Arabidopsis* HDA19-RNAi lines showed a normal phenotypes after germination (Tanaka et al., 2008). However, HDA6/HDA19-RNAi double mutants displayed severe arrested post-germination growth and formation of embryo-like structures on the true leaves of 6-week-old plants, even without TSA treatment. In addition, the embryonic expression of *FUS3* and *ABI3* were maintained in seedlings of the double knockdown plants 7d after sowing (Tanaka et al., 2008). This indicates that the two HDACs have a redundant function in controlling plant developmental transition.

More recent results showed that HDA19 interacts with the HIGH-LEVEL EXPRESSION OF SUGAR-INDUCIBLE GENE2-LIKE1 (HSL1) to regulate seed maturation gene expression in seedlings (Zhou et al., 2013). HSL1 is EAR-motif-containing transcriptional repressor that acts redundantly with other regulators to repress the ectopic expression of seed maturation genes in seedlings via sugar signaling (Suzuki et al., 2007; Tsukagoshi et al., 2007; Tsukagoshi et al., 2005). Loss-of-function mutants of *HSL1* displayed increased acetylation of histone H3 and H4 and elevated transcription of certain seed maturation genes in seedlings, which was also observed in *hda19* mutants (Zhou et al., 2013). Moreover, ChIP assays

showed that HDA19 binds directly to the chromatin of seed maturation genes (Zhou et al., 2013). These data together suggest that HSL1 may repress seed maturation gene expression in seedlings through HDA19-mediated histone deacetylation. In addition, a nuclear GRAS (GIBBERELLIN ACID INSENSITIVE, REPRESSOR OF GIBBERELLIC ACID INSENSITIVE3 and SCARECROW [SCR]) protein, SCR-LIKE15 (SCL15), was also found to specifically interact with HDA19 and play a crucial role in deacetylation and repression of genes associated with seed maturation. *scl15-1* seedlings ectopically express a large subset of embryonic genes in seedlings, which show high levels of histone H3 acetylation (Gao et al., 2015). It is therefore proposed that SCL15 acts as part of HDA19-associated transcriptional repressor complex to repress the expression of embryonic genes in seedlings.

The *hda19* loss-of-function lines also displayed defects in vegetative and reproductive development including shorter plant height, aberrant leaves, abnormal flowers, and reduced female fertility (Tian and Chen, 2001; Tian et al., 2005; Zhou et al., 2005). Several studies suggested that HDA19 is directly recruited to TOPLESS (TPL) to form a transcriptional co-repressor complex to actively repress gene expression (Long et al., 2006; Ryu et al., 2014). It was shown that APETALA2 interacts with the TPL/HDA19 co-repressor complex to control the outer expression boundaries of B-, C- and E-class genes of floral organogenesis (Krogan et al., 2012). Moreover, experimental data showed that brassinosteroid-activated BES1 also recruits the TPL-HDA19 co-repressor complex to inhibit abscisic acid (ABA) responses in early seedling by repressing *ABI3* expression (Ryu et al., 2014).

Another RPD3 class I member, HDA9, is also shown to be involved in control of vegetative and flower development in *Arabidopsis* (Kang et al., 2015; Kim et al., 2013; van Zanten et al., 2014). Kim and colleagues firstly demonstrated that loss-of-function mutations of *HDA9* led to early flowering in short days and derepression of AGAMOUS-LIKE 19 (AGL19) that promotes flowering through a FLC independent pathway (Kim et al., 2013). Kang et al further proved that HDA9 protein directly targets upstream promoter regions of *AGL19* and regulates its histone acetylation and controls expression of the floral integrator *FLOWERING LOCUS T* (*FT*) and flowering (Kang et al., 2015). Thus, HDA9 has a function to prevent precocious flowering under short day conditions by controlling AGL19 expression

through histone H3 deacetylation. Interestingly, HDA9 was proposed to possess an opposite function of HDA6 and HDA19 in controlling early seed development and seedling growth in *Arabidopsis* (van Zanten et al., 2014). The *hda9* mutation significantly reduced seed dormancy and led to earlier seed germination than wild-type plants. Additionally, by comparison of transcriptomes between a *hda9* mutant and wild-type, several genes associated with photosynthesis and photoautotrophic growth such as those encoding RuBisCO and RuBisCO activase were found to be prematurely expressed in *hda9-1* dry seeds, resulting in premature initiation of imbibition program in *hda9-1* seeds. ChIP experiments further demonstrated enhanced H3K9 acetylation levels at the promoters and transcribed regions of these photosynthesis-regulated genes in young *hda9-1* seedlings (van Zanten et al., 2014). It is therefore suggested that HDA9 negatively regulates seedling traits in dry seeds, probably by transcriptional repression via histone deacetylation.

In addition, another class II member, AtHDA15 (At3g18520), was found to play important roles in a wide range of light-dependent plant developmental processes (Gu et al., 2017; Liu et al., 2013; Tang et al., 2017). In the presence of light, AtHDA15 is imported into the nucleus, but is exported out of the nucleus in the dark (Alinsug et al., 2012), implying that its function is possibly regulated by light. A previous study provided evidence that PHYTOCHROME INTERACTING FACTOR3 (PIF3) recruits HDA15 to repress light-responsive genes expression by reducing H4 acetylation levels in the dark, thus, resulting in the inhibition of chlorophyll biosynthesis and photosynthesis. The interaction of PIF3 and HDA15 is dissociated under red light condition (Liu et al., 2013). In the dark condition, HDA15 was also found to interact with PIF1 that co-regulate the expression of light-responsive genes associated with hormonal signaling pathways and cellular processes in germinating seeds (Gu et al., 2017). Interesting, Tang et al. recently showed that HDA15 physically interacts with NUCLEAR FACTOR-Y C proteins to mediate the hypocotyl elongation in a light-dependent manner (Tang et al., 2017). Together, these data suggest that through interaction with transcription factors, HDA15 regulates light-stimulated developmental processes via histone deacetylation.

1.1.4.3.2 Functions of RPD3/HDA1 family HDACs in plant stress response

A number of studies have shown that several HDACs or multiprotein complex formed by HDACs contribute to plant responses to various biotic stresses. HDA19 was found to play a role in integrating histone acetylation with ethylene and jasmonic acid (JA) signals to modulate the expression of stress responsive genes (Zhou et al., 2005). Overexpression of *HDA19* in transgenic plants significantly induced the expression of *ETHYLENE RESPONSE FACTOR1* (*ERF1*) and ethylene- and JA-regulated *PR* genes, and resulted in enhanced resistance to pathogen *A. brassicicol*. Consistently, HDA19-RNAi plants displayed opposite phenotypes and decreased expressions of corresponding downstream genes (Zhou et al., 2005). In addition, experimental data revealed physical interaction between HDA19 and two WRKY transcription factors (WRKY38 and WRKY62) that are negative regulators of plant basal defense (Kim et al., 2008). Expression of *HDA19* was induced by *P. syringae* and loss of HDA19 activity weakened the resistance, whereas overexpression of *HDA19* enhanced resistance to *P. syringae*. *HDA19* over-expression also reduced the transcriptional regulatory activity of WRKY38 and WRKY62 in plant cells (Kim et al., 2008), suggesting an opposite role between HDA19 and these two transcription factors in basal resistance to the bacterial pathogen. Thus, interaction of WRKY38 and WRKY62 with HDA19 may act to fine-tune plant basal defense responses.

In addition to function in resistance response to biotic stresses, HDACs were also found to be involved in abiotic stress response. For example, HDA19-regulated histone deactylation is involved in temperature regulation of plant development. *hda19-1* mutants displayed temperature-sensitive phenotypes including disorganized root and shoot meristems to form pin or tubular or single cotyledon when grown at 29 °C but not at room temperature (25 °C) (Long et al., 2006). In addition, *hda6* mutant plants showed a freezing-sensitive phenotype after cold-acclimation (To et al., 2011). The expression of several cold stress induced genes was found to be regulated by HDA6, suggesting that the cold acclimation process may be regulated by HDA6-mediated histone deacetylation. Involvement of histone acetylation in plant responses to other biotic stresses has been also documented. For example, HDA9 negatively regulates gene expression in response to salt and drought stress (Zheng et al., 2016). Compared with wild-type plants, T-DNA insertion mutant lines of *AtHDA9*

(*hda9-1* and *hda9-2*) displayed insensitivity to both NaCl and polyethylene glycol (PEG) treatment during germination and increased tolerance to salt and drought stress. RNA sequencing analysis showed that a number of genes involved in respond to water deprivation stress, temperature and light stimuli, etc., are up-regulated in *hda9-2* mutant (Zheng et al., 2016), suggesting a general role of HDA9 in salt and drought stress responses. ChIP analysis further revealed that in the *hda9* mutants, salt and drought stresses resulted in much higher levels of histone H3K9 acetylation at promoters of water-deprivation stress-related genes than in wild-type plants (Zheng et al., 2016). Although it is not yet determined whether stress-related transcription factors and signaling proteins interact with HDA9, HDA9-mediated histone deacetylation is likely to modulate the expression of a large number of stress-responsive genes.

1.2 Interplay between salicylic acid and redox signaling in the control of plant stress responses

1.2.1 The functions of SA in plant development and immunity

Plant hormones, or phytohormones, are a class of small, organic molecules that are not only essential for the regulation of plant growth and development, but also play an integral role in both abiotic and biotic stress responses (Bari and Jones, 2009; Pieterse et al., 2009; Verma et al., 2016). In turn, different biotic and abiotic stress conditions can influence the hormone concentration or sensitivity, which mediates a whole range of adaptive plant responses (Takacs et al., 2016). Among them, SA has been shown to act as a versatile regulator that plays crucial roles in many development pathways and responses to environmental conditions, including seed germination, seedling establishment, plant cell growth, respiration, stomatal aperture, leaf senescence-associated gene expression, basal thermotolerance, responses to abiotic stresses, fruit yield and nodulation in legumes, etc., throughout the entire lifespan of the plant **(Fig 7)** (Clarke et al., 2004; Kang et al., 2012; Martinez et al., 2004; Mateo et al., 2004; Metwally et al., 2003; Morris et al., 2000; Norman et al., 2004; Rajjou et al., 2006; Rate et al., 1999). Its effect on these processes may be either direct or indirect because SA also influences the synthesis and/or signaling of other plant hormones such as JA, ET, and auxin (Boatwright and Pajerowska-Mukhtar, 2013). In addition, SA functions in a dose-dependent manner, where plant functions can be possible induced by low SA concentration but inhibited by high SA concentration. For instance, in *Matricaria chamomilla*, 50 μM SA were found to promote growth but an inhibited growth was evidenced with 250 μM (Kovácik et al., 2009). Apart from the concentration, the effects of SA in plants can be also influenced by the duration of the treatment, plant species, age and treated plant organ (Miura and Tada, 2014).

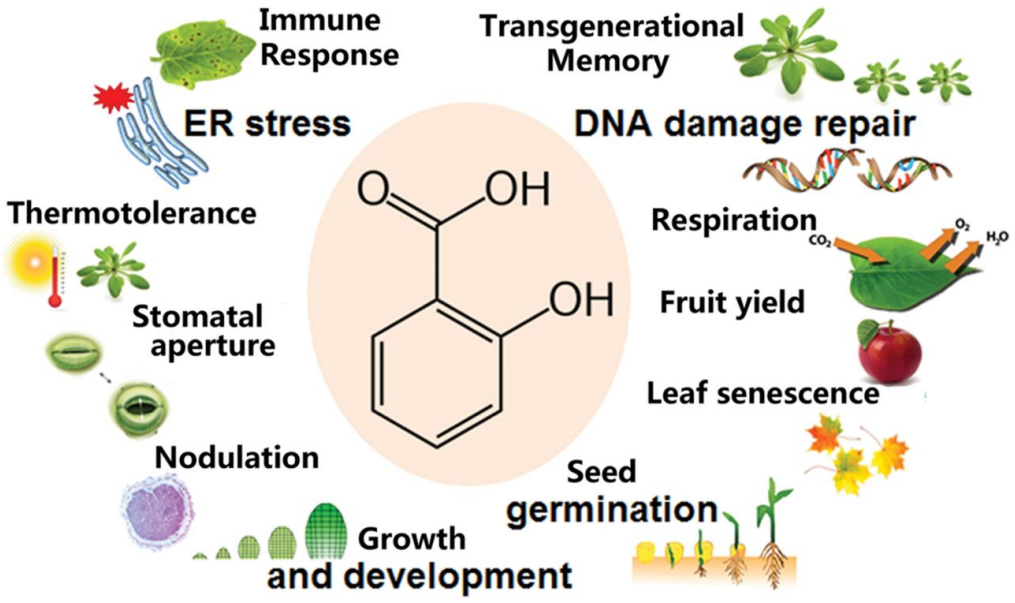

Figure 7. Biological functions of salicylic acid in plants. Salicylic acid (SA) plays essential roles in the regulation of diverse developmental processes and stress responses in plants. Along with the well-established functions of SA in plant immune responses, plant cell growth, seed germination, thermotolerance, respiration, stomatal aperture, fruit yield, nodulation and leaf senescence, novel functions are coming to be understood, including roles in endoplasmic reticulum stress, DNA damage repair and transgenerational memory (Liu et al., 2015b).

1.2.2 SA biosynthesis

SA can be generated via two distinct biosynthesis pathways in plants that require the primary metabolite chorismate produced through shikimate pathway, namely the isochorismate (IC) pathway and the phenylalanine ammonia-lyase (PAL) pathway **(Fig 8)** (Chen et al., 2009; Wildermuth, 2006). Although these two SA biosynthesis pathways are well defined, several questions such as their mechanisms and relative contributions have not been yet fully understood. It is generally believed that only

small percentage of SA is produced through the PAL pathway, where SA is formed from chorismate-derived L-phenylalanine via a series of enzymatic reactions initially catalyzed by PAL (Huang et al., 2010). In contrary, the IC pathway is thought to product the majority of SA in plants (An and Mou, 2011). In this synthesis pathway, chorismate is converted into SA in a two-step process metabolized by the enzymes ISOCHORISMATESYNTHASE (ICS), which converts chorismate to IC, and ISOCHORISMATE PYRUVATE LYASE (IPL), which catalyzes the conversion of IC into SA (Chen et al., 2009). *Arabidopsis* contains two ICS genes (*ICS1* and *ICS2*); ICS1 (also known as SALICYLIC ACID INDUCTION DEFICIENT 2 SID2) is likely to play a dominant role in pathogen-induced SA biosynthesis because SA accumulation, as well as pathogen resistance, was severely compromised in mutant plants lacking functional ICS1 (*sid2*/*eds16*) during pathogenic interactions (Catinot et al., 2008; Strawn et al., 2007). Additionally, the expression of *ICS1*, but not of *ICS2*, was induced by a range of biotic and abiotic stresses, such as pathogen infection, detection of microbe-associated molecular patterns and SA treatments, as well as UV and ozone exposure (Catinot et al., 2008; Verberne et al., 2000; Wildermuth et al., 2001), further indicating that ICS1 is required for the majority of stress-induced SA biosynthesis. *ICS2* can also encode a functional ICS enzyme but in limited amounts (Garcion et al., 2008). The appearance of residual SA in an *ics1*/*ics2* double mutant confirmed the existence of an alternative route to SA biosynthesis in *Arabidopsis* (Mauch-Mani and Slusarenko, 1996). SA is synthesized in the chloroplasts and most of them are readily converted into its biologically inactive form, SA β-glucoside (SAG), by a pathogen-inducible SA GLU-COSYLTRANSFERASE in the cytosol (Lee and Raskin, 1999). SAG is then actively transported to the vacuole, where it acts as an inactive storage until conversion back to biologically active SA (Dean and Mills, 2004).

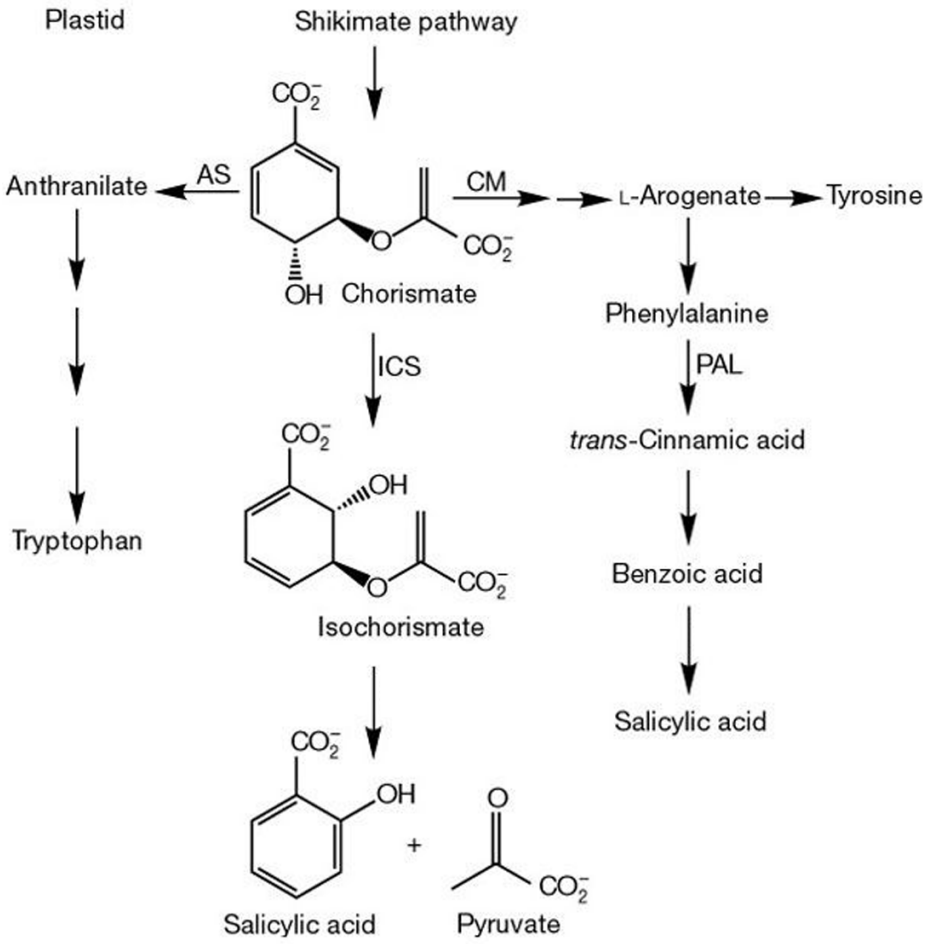

Figure 8. Proposed pathway for SA biosynthesis in plants. AS, anthranilate synthase; CM, chorismate mutase; ICS, isochorismate synthase; PAL, Phe ammonium lyase (adapted from Wildermuth et al., 2001).

1.2.3 Involvement of SA in redox-signaling and oxidative protein modifications

SA-mediated signaling pathways have been found as regulators of defense responses through their interplay with several other signaling pathways including redox signaling. These processes induce a set of molecular events that involve SA synthesis, the production of reactive oxygen (ROS) and nitrogen (RNS) species, as well as

redox-based protein modifications, finally resulting in the expression of pathogen resistance genes (Mammarella et al., 2015; Overmyer et al., 2003; Spoel and Loake, 2011; Vlot et al., 2009; Xia et al., 2015; Yan and Dong, 2014; Zheng et al., 2015; Zottini et al., 2007).

1.2.3.1 Redox signaling

Redox chemistry is an intrinsic part of plant metabolism. Challenged by oxidants from chloroplasts, peroxisomes, mitochondria, and apoplasts, plants have evolved sophisticated mechanisms to control ROS/RNS, as well as their damaging properties (Asada, 2006; Brautigam et al., 2009; Daudi et al., 2012; Puerto-Galan et al., 2015; Rhoads et al., 2006; Suzuki et al., 2011). The redox homeostasis is controlled mainly by the thioredoxin (Trx)/peroxiredoxin (Prx) and glutathione (GSH) systems, where the electron flow or redox flux is established from lower to higher reduction potentials (Davletova et al., 2005; Geigenberger and Fernie, 2014; Noctor et al., 2015; Sandalio and Romero-Puertas, 2015). The alteration in steady-state level of ROS/RNS and subsequent changes in cellular redox state are important systems to regulate cellular signaling factors linking environmental stimuli with intracellular signal transduction pathways in response to stresses (Baxter et al., 2014; Camejo et al., 2016; Sierla et al., 2013). ROS, including the hydroxyl radical (●OH), superoxide ($O2^{\bullet-}$), singlet oxygen ($^1O_2$), and hydrogen peroxide (H_2O_2), and RNS, deriving from nitric oxide (NO) and $O2^{\bullet-}$, are able to react with a broad range of biomolecules (Gechev et al., 2006). Recent studies showed that ROS/RNS likely play their roles through oxidative modifications of proteins that are sensitive to perturbation of cellular redox states (Chi et al., 2013; Rinalducci et al., 2008). Owing to the reactivity of their thiol groups, some protein cysteine (Cys) residues make them excellent sites for oxidative signaling. Indeed, several reversible Cys modifications with increasing oxidative levels have been characterized, such as S-nitrosylation (SNO, i.e. the covalent attachment of NO), S-sulphenation (SOH, i.e. thiol hydroxylation), S-sulphination (SO_2H, i.e. further oxidation of SOH) and S-sulfonation (SO_3H, i.e. further oxidation of SO_2H) **(Fig 9)** (Astier et al., 2012; Mieyal and Chock, 2012; Waszczak et al., 2015). Such oxidative modifications in thiol-containing enzymes have now been shown to modulate their structure and function, providing the missing link between hormone-induced redox

perturbations and downstream protein action.

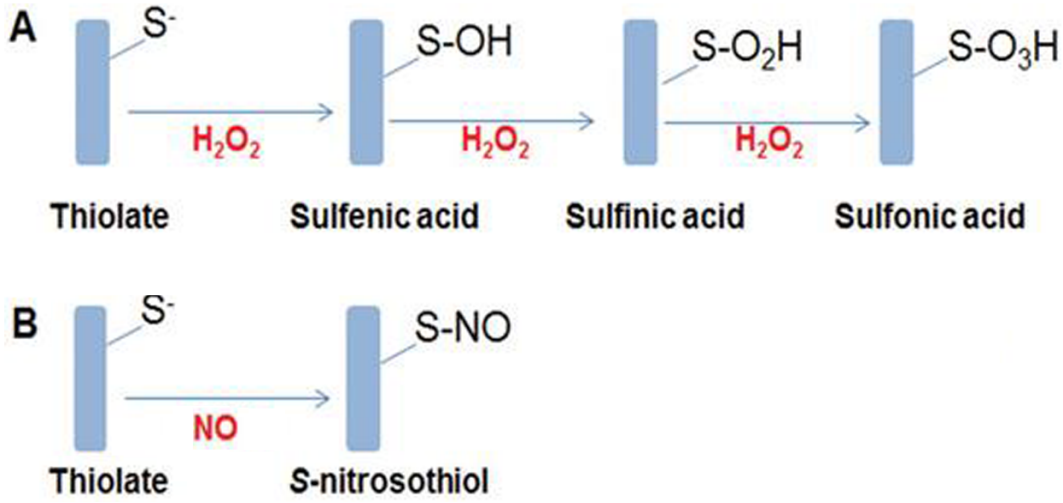

Figure 9. Commonly observed cysteine (Cys) oxidative modifications by ROS/RNS. (A) Different oxidation of Cys is shown. Exposure of free Cys thiols (S^-) to an increasingly oxidized environment results in reversible S-sulphenation (SOH), S-sulphination and S-sulfonation. (B) Protein S-nitrosylation by reaction of the thiolate form with nitric oxide (adapted from Sevilla et al., 2015).

1.2.3.2 Cross-talking between SA- and redox- signaling

The interactions between SA and ROS are complicated in plants. Several observations lead to the hypothesis that ROS and SA form a self-amplifying feedback loop, in which ROS induce SA synthesis and SA subsequently enhances ROS accumulation (Vlot et al., 2009; Yang et al., 2015). Simply speaking, upon recognition of pathogen infection, one of the earliest host responses is increased production of ROS such as $O2^{\bullet-}$ and H_2O_2 in the apoplast (Maruta et al., 2012), which occurs within several minutes of infection. Although such increased ROS associated with the oxidative burst is weak and transient, it is believed to play an important role in activation of the plant immune response (Gadjev et al., 2006). The altered ROS signaling initiated in pathogen infection stimulates synthesis of SA. Simultaneously, increased levels of SA

disturb cellular redox homeostasis by inhibiting catalase in the peroxisomes (Geigenberger and Fernie, 2014), producing sustained oxidative burst to potentiate cell death and defense gene expression, which also in turn activates the synthesis of more SA.

The relationship between NO, SA, and SA-mediated signaling pathways in the activation of defense genes is well defined in *Arabidopsis*. It was proposed that NO and SA are likely to function in a positive feedback loop (Zottini et al., 2007). Studies have suggested that SA can induce NO production in part through a NO synthase-dependent pathway and in turn, NO is involved in the regulation of SA biosynthesis and most importantly, plays essential roles in SA-mediated defense response (Saleh et al., 2015; Zottini et al., 2007). NON-EXPRESSOR OF PR GENES 1 (NPR1) has been identified as an important transducer of the SA signal, in which, the protein structure and functions of NPR1 were tightly modulated by SA-mediated NO singling (Mou et al., 2003; Tada et al., 2008; Zhou et al., 2015). NPR1 contains several conserved Cys residues that establish a high molecular weight NPR1 oligomer through the formation of intermolecular disulphide bridges. This stable structure makes the NPR1 protein confine at the cytoplasm (Mou et al., 2003). Upon pathogen infection, SA triggers perturbation of cellular reduction and oxidation that result in disulphide reduction of NPR1 oligomer at the residues Cys82 and Cys216 by Trx-NADPH system. Disulphide reduction releases NPR1 monomer that translocates to the nucleus (Mou et al., 2003; Tada et al., 2008). Interestingly, this activation process is also accompanied with oligomerization of NPR1 promoted by S-nitrosylation of Cys156, providing an antagonistic interplay between these two modifications (Tada et al., 2008). S-nitrosylation of NPR1 facilitates the formation of disulphide bonds, which is essential to maintain NPR1 abundance and homeostasis upon SA induction.

1.2.4 Interaction of SA with HDACs

Chromatin modification has recently been shown to be involved in regulation of SA-mediated plant defense responses in *Arabidopsis*. Loss of HDA19 activity was

found to enhance pathogen resistance as a result of increased SA content and induced expression of a set of genes required for accumulation of SA that eventually activated the NPR1-dependent stress response process and expressed PR genes (Choi et al., 2012). It was further demonstrated that HDA19 directly targets the promoter regions of *PR1*, *PR2*, and *GDG1* (*GH3-LIKE DEFENSE GENE*, one of key components for SA accumulation), which represses their expression under unchallenged conditions by histone deacetylation (Choi et al., 2012). Thus, HDA19 is most likely required for the repression of SA biosynthesis and SA-mediated defense responses. Conversely, SA-mediated signaling pathways were also shown to modulate the functions of HDACs (Liu et al., 2015a; Mengel et al., 2017). It was observed that HDA9 and HDA19 undergo oxidative Cys modifications in response to SA, as demonstrated in a proteomic analysis aimed to identify early redox-regulated proteins in the defense response (Liu et al., 2015a). The oxidation of HDA19 and HDA9 could affect their conformation, location or activity, leading to changes in the levels of histone acetylation and expression of associated genes. Interestingly, Mengel et al. recently showed that S-nitrosoglutathione (GSNO), a physiological NO donor, as well as SA-mediated NO signaling inhibits cellular HDAC activity *in vivo* and increases global H3 and H4 acetylation, providing a novel role of NO during plant-pathogen interactions (Mengel et al., 2017). Moreover, changes in global histone acetylation levels affected by INA (2,6-dichloro-isonicotinic acid, functional analog of SA) were found to be smaller than that of GSNO treatment (Mengel et al., 2017), indicating that endogenously produced NO might selectively target a subset of NO-sensitive HDACs. Together, these data initially establish a regulatory network within SA signaling pathways and chromatin modification, where SA-mediated NO signaling may inactivate specific HDACs through redox modifications under biotic/abiotic stress, resulting in enhanced histone acetylation and a supportive chromatin state for the expression of defense-related genes **(Fig 10)**.

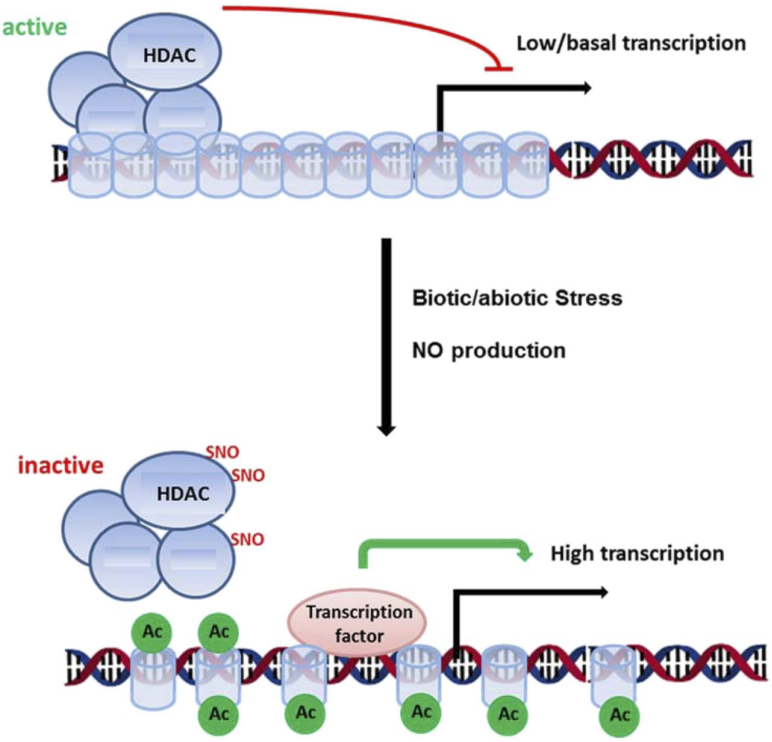

Figure 10. SA-mediated NO signaling inhibits HDACs activity that contributes to stress gene induction. In unchallenged conditions, the transcription of defense related genes is repressed by HDAC complexes through deacetylation of the corresponding chromatin regions. Under biotic/abiotic stress, NO is produced, resulting in the inactivation of HDAC complexes. Opened chromatin state facilitates the genes transcription. Blue cylinders, Nucleosomes; green circles, acetyl groups (Ac); black arrow, TSS (Mengel et al., 2017).

1.3 Thermomorphogenesis signaling network in plants

1.3.1 Temperature, plant development, and thermomorphgenesis

Plants have evolved sophisticated regulatory mechanisms to adapt quickly to their unfavorable surrounding environment such as heat, cold, drought, and pathogen infections. In particular, temperature, as a key environmental variable, greatly affects plant growth and development and has dramatic effects on plant architecture and biomass throughout the entire lifecycle (Atkin et al., 2006; Patel and Franklin, 2014). For example, in many species, plant seed germination has shown to be regulated by temperature (Penfield et al., 2005). A prolonged period of cold (stratification) promotes seed germination, whereas seeds germination is inhibited by high temperature treatment (Toh et al., 2008). It was also observed that within the range of about 12-27 °C, plants exhibit dramatic differences without significant induction of stress responses, in terms of growth rates, phenotypes, and developmental responses (Atkin et al., 2006), indicating the leading roles of temperature in plant development. Moreover, elevations in ambient temperature can accelerate flowering time in *Arabidopsis*, resulting from induction of the floral integrator FLOWERING TIME (Balasubramanian et al., 2006; Blazquez et al., 2003; Lee et al., 2007; McClung et al., 2016).

Projected global warming poses a serious threat to agricultural productivity since even small increases in ambient temperature considerably hampers plant productivity (Lobell and Field, 2007). Thus, uncovering the temperature sensing mechanisms in plants becomes urgent and, most importantly, is crucial for providing molecular and functional insight that could help to maintain productivity in a changing climate. Experimentally speaking, for the model plant *Arabidopsis*, heat is generally classified into three levels: warm ambient temperature (about 20-27 °C), high temperature (27-30 °C), and extremely high temperature (37-42 °C, also known as heat stress). Although the responses to extremes of temperature on plants have been studied for many decades, the mechanisms in how plants cope with moderate increased, non-stress responded temperature change (also referred to as warm ambient

temperature) are only beginning to understand. In fact, several plant species are found to have the potential to acclimate to warm ambient temperature by changing their architecture (Franklin, 2009; Samach and Wigge, 2005). These plants show adaptive elongation growth of stems (hypocotyl) and leaves to enhance cooling capacity and avoid heat flux **(Fig 11)** (Quint et al., 2016). This acclimation process is called thermomorphogenesis and is critical for plant fitness and yield. Comprehensive understanding of the molecular networks controlling thermomorphogenesis is therefore pivotal to develop next-generation climate warming-resistant crop cultivars.

Figure 11. Typical thermomorphogenesis phenotypes of *Arabidopsis thaliana* plants. Artist impression of thermomorphogenesis phenotypes of (a) young *Arabidopsis* seedling (7 days-old) and (b) vegetative plants (30 days-old), grown at control temperature (20 °C) or high ambient temperature (28 °C). Note the occurrence of enhanced elongation growth of hypocotyls (embryonic stem) and petioles (leaf stalk), as well as hyponasty of petioles and leaves (upward leaf movement) in both seedlings and vegetative plants. Together these adaptations result in an "open" rosette structure that facilitates leaf-cooling capacity (Quint et al., 2016).

1.3.2 Association between light and temperature signaling network

Light and temperature patterns being the most dynamic parameters are often correlated under normal plant growth environment. It is well known that both light and temperature strongly influence seedling hypocotyl elongation, which makes it as a key trait of great adaptive significance (Arsovski et al., 2012; Penfield, 2008). At

constant temperature condition, elongation of seedling hypocotyl is rapid in darkness and is inhibited by light. Correspondingly, under normal light condition, warm ambient temperature promotes seedling hypocotyl elongation, whereas lower temperatures inhibit elongation growth of hypocotyl (Franklin, 2009; Franklin et al., 2014). While the regulatory mechanisms for sensing light are well established in plants, those required for perception and response to changes in ambient temperature still remain poorly understood.

Owing to the fact that light and temperature conditions are not independent at natural environment, some signaling pathways shared by these two environmental cues are more likely than others. Interestingly, recent studies that focused on thermosensory growth identified several key factors with a defined role in light signaling (Legris et al., 2017; Lorenzo et al., 2016), further highlighting the tight coordination of light signaling and temperature responses. Among these factors, the bHLH transcriptional regulator PHYTOCHROME INTERACTING FACTOR 4 (PIF4) has emerged as a central signaling hub for the integration of light quality and temperature (Koini et al., 2009; Kumar et al., 2012; Leivar and Monte, 2014; Leivar and Quail, 2011; Paik et al., 2017; Proveniers and van Zanten, 2013). Previous evidences showed that PIF4 acts as a key regulator of photomorphogenic development that promotes stem growth (Leivar and Quail, 2011), whilst *pif4* mutants are unable to increase hypocotyl length at warmer temperatures (Koini et al., 2009); PIF4 is therefore shared by growth responses to light and temperature. The activity and/or protein levels of PIF4 are originally shown to be controlled by photoreceptor phytochrome B (phyB) in a light-dependent manner (Park et al., 2012). phyB is interconvertible photoreceptor that switches between an inactive Pr state and an active Pfr state upon absorbing red and far-red light, respectively (Legris et al., 2016). Light-activated phyB interacts with PIF4, which accelerates the degradation of PIF4 partially in the 26S proteasome and/or affects binding capacity of PIF4 to target gene promoters (Jung et al., 2016). However, apart from its photoreceptor functions, phyB was recently found to also act as a temperature sensor in plants, where warm ambient temperature increases the reversion rate from the active Pfr state to the inactive Pr state (Jung et al., 2016). Hence, elevated ambient temperature attenuates negative role of phyB in PIF4-mediated promotion of hypocotyl elongation, resulting in increased hypocotyl length. Moreover, the gene expression of *PIF4*, as well as protein stability, is also

increased under warm ambient temperatures (Delker et al., 2014), suggesting that additional regulatory components contribute to defining the PIF4-medaited thermosensory growth.

Another interconnected branch, DE-ETIOLATED 1 (DET1)-CONSTITUTIVE PHOTOMORPHOGENIC 1 (COP1)-ELONGATED HY5-dependent photomorphogenesis signaling pathway, was defined to be involved in PIF4-mediated thermosensory elongation growth. COP1 is a RING E3 ligase that directly targets HY5, a negative regulator of elongation growth, for degradation in the 26S proteasome in the dark (Osterlund et al., 2000). Although COP1 can function on its own *in vitro*, *in vivo* it need to form a complex with SUPPRESSOR OF PHYA-105 1 (SPA1), SPA2, SPA3 and/or SPA4 (COP1-SPA) (Zhu et al., 2008). DE-ETIOLATED 1 (DET1) assembles with COP10 and DAMAGED DNA BINDING PROTEIN 1 (DDB1) into the CULLIN 4 (CUL4)-based E3 ubiquitin ligase, forming a multimeric CUL4-DDB1$^{COP10\text{-}DET1}$ (CDD) (Lau and Deng, 2012). The COP1-SPA core acts in turn as a substrate adaptor for the CDD complex that is thought to apparently reinforce activity of the CDD-COP1-SPA multimeric complex (Lau and Deng, 2012). It has shown that stability of PIF4 protein is maintained by both COP1 and DET1, as very low levels of PIF4 protein are observed in *cop1* and *det1* mutants (Delker et al., 2014). Moreover, the *cop1*, *det1*, *cop10*, *cul4* and *spa1 spa3 spa4* mutants displayed a reduced hypocotyl growth phenotype and the expression of *PIF4* (Delker et al., 2014; Park et al., 2017), as well as its temperature-responsive genes, was strongly downregulated in *det1-1* mutants in warm temperatures (Delker et al., 2014; Gangappa and Kumar, 2017). Thus, DET1/COP1 most likely acts as the core positive regulatory module for PIF4-mediated thermosensory growth. Another point of regulatory network in PIF4-mediated elongation growth arises from the inhibition of HY5 function by DET1/COP1 (Gangappa and Kumar, 2017). It is known that HY5 competitively occupies G-box elements of co-targeted promoter regions with PIF4 and represses the downstream genes expression of PIF4 (Gangappa and Kumar, 2017). Continuous warm temperatures increase the nuclear abundance of COP1 and PIF4 and reduce that of HY5 (Gangappa and Kumar, 2017; Park et al., 2017). Hence, it seems that increased temperature results in significantly reducing this competitive inhibition through decreasing the accumulation of HY5 protein (most likely due to enhanced activity of COP1), as well as *HY5* gene expression level.

Together, although the list is by no means comprehensive, current information provides a PIF4-mediated mechanistic framework where multiple regulatory components cooperatively control plant growth through integrating light and temperature signal **(Fig 12)**.

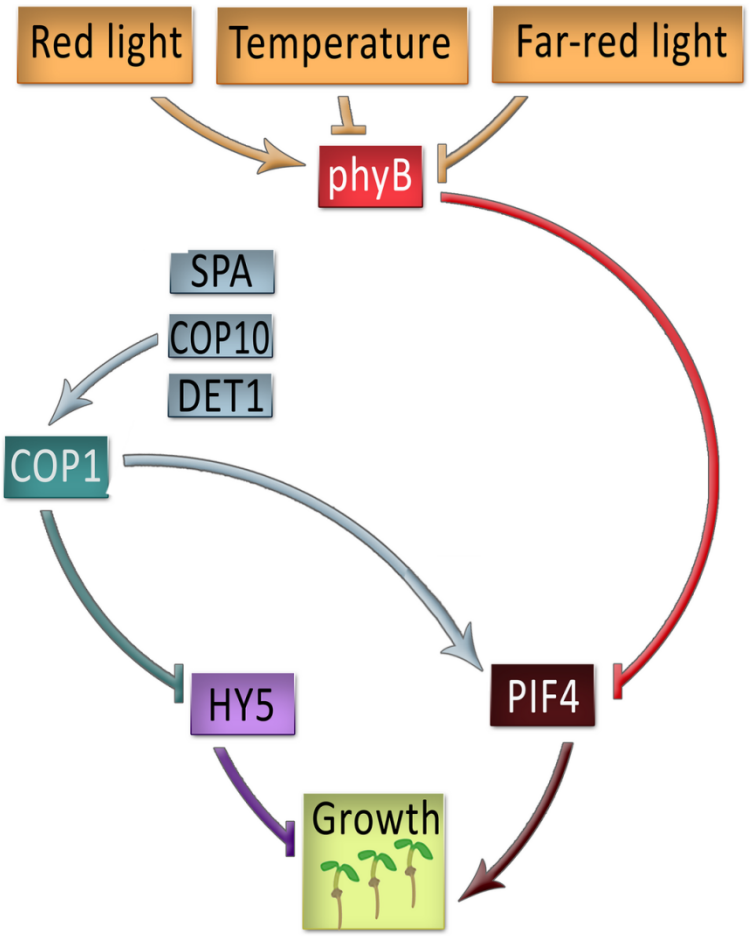

Figure 12. PIF4-mediated thermosensory signaling that control growth. The scheme highlights key components of two interconnected signaling branches. COP1, CONSTITUTIVE PHOTOMORPHOGENIC 1; SPA, SUPPRESSOR OF PHYA-105 1; DET1, DE-ETIOLATED1; HY5, ELONGATED HYPOCOTYL 5; PIF4, PHYTOCHROME INTERACTING FACTOR 4 (Adapted from Legris et al., 2017).

1.3.3 Integration of temperature signaling and hormones

Two major phytohormones, auxin and BR, were found to be involved in warmer temperatures-induced elongation growth of hypocotyl in *Arabidopsis* (Stavang et al., 2009; van Zanten et al., 2009). It was observed that the accumulation of free auxin increases with elevated temperature, triggering the cell elongation responses that result in hypocotyl elongation (de Wit et al., 2014; Gray et al., 1998). In contrary, high temperature-mediated hypocotyl elongation is abolished in auxin signaling mutants (Franklin et al., 2011). Transcriptomic analysis of high temperature responses also revealed that auxin-responsive genes are strongly enriched among the induced genes, such as indole-3-acetic acid (*IAA*) 19 and *IAA29* (auxin signaling), pin-formed3 (*PIN3*) and *PIN7* as well as small auxin up RNA (*SAUR*) 19-24 (auxin transport) (Hornitschek et al., 2012), suggesting the key role of auxin for thermosensory growth. Additionally, recent studies provided evidence that three auxin biosynthesis genes, *TRYPTOPHAN AMINOSTRANSFERASE OF ARABIDOPSIS1 (TAA1*), *CYTOCHROME P450, FAMILY 79, SUBFAMILY B, PEPTIDE 2* (*CYP79B2*) and *YUCCA8* (*YUC8*), are upregulated by higher temperature in a PIF4-dependent manner as high temperature-induced upregulation of their expression was largely suppressed in *pif4* seedlings (Hornitschek et al., 2012; Sun et al., 2012). ChIP analysis further demonstrated that PIF4 directly binds to E-box and/or G-box motif in their promoters (Sun et al., 2012). Moreover, exogenous application of synthetic auxins largely rescued the impaired high temperature-induced hypocotyl elongation in *pif4* mutants (Sun et al., 2012). It is therefore suggested a direct mechanism by which PIF4 controls higher temperature-induced hypocotyl elongation by stimulating auxin biosynthesis.

BR signaling has been shown to be essential for temperature-mediated hypocotyl elongation. Seedlings deficient in BR biosynthesis or signaling or treated with the BR biosynthesis inhibitor brassinazole displayed a greatly inhibition of warm temperature-induced hypocotyl elongation (Oh et al., 2012). At the molecular level, BRs regulate gene expression and growth through the control of the phosphorylation status of the BRASSINAZOLE-RESISTANT transcription factors 1 (BZR1) and BZR2. The increased BR levels promote the dephosphorylation of BZR1 and BZR2 that makes them active, leading to the BR-regulated gene expression (Belkhadir and

Jaillais, 2015; Yamada et al., 2009). Interestingly, BZR1 was found recently to directly interact with PIF4 and co-regulate expression of many key target genes with roles in cell elongation synergistically (Oh et al., 2012). It has also shown that BR synthesis is required for promotion of hypocotyl growth in the warm temperatures, not only for accumulation of active BZR1 but also to stabilize PIF4 (Bernardo-Garcia et al., 2014). Although BZR1 is needed for hypocotyl elongation at warm temperatures, its level is not significantly affected by temperature (Oh et al., 2012).

1.3.4 Epigenetic control of plant ambient temperature responses in *Arabidopsis*

As mentioned above, plants have developed a complex regulatory network involved in reprogramming of gene expression closely responding to environmental changes via epigenetic mechanisms. Recently, an important study revealed that chromatin has a key role in the perception of changes in ambient temperature **(Fig 13)**. Histone variants are non-canonical (non-allelic) variants of histones, which have been shown to play important roles in maintenance of genome stability, transcriptional activation and repression by altering the properties of the nucleosomes and affecting chromatin remodeling (To and Kim, 2014). In a forward genetic screen of mutants having mis-regulated *HSP70::LUC*, the *ACTIN RELATED PROTEIN6 (ARP6)* gene was identified to coordinate the response to differences in ambient temperature (Kumar and Wigge, 2010). The APR6 protein is an essential component of the SWR1 chromatin-remodeling complex that is required for histone variant H2A.Z incorporation into nucleosomes (Deal et al., 2007; Kobor et al., 2004; Krogan et al., 2003; Mizuguchi et al., 2004). When grown at 22 °C, *arp6* mutants displayed similar phenotypes, such as hypocotyl and petiole elongation, leaf hyponasty, and early flowering, to that of wild-type plants grown at 27 °C, and transcriptomes analysis revealed that warm temperature transcriptome is constitutively expressed in *arp6-10* mutants that compromise H2A.Z occupancy at lower temperature (Kumar and Wigge, 2010). Additionally, ChIP experiments showed that H2A.Z occupancy at promoter regions of related-genes dramatically decreased with warm temperature independently of transcriptional response (Kumar and Wigge, 2010). It is therefore suggested that

H2A.Z occupancy represses gene expression by creating a physical block to transcription or by preventing the binding of transcription activators at lower temperatures, and eviction of H2A.Z-containing nucleosomes at higher temperatures would thereby facilitate transcription of target genes.

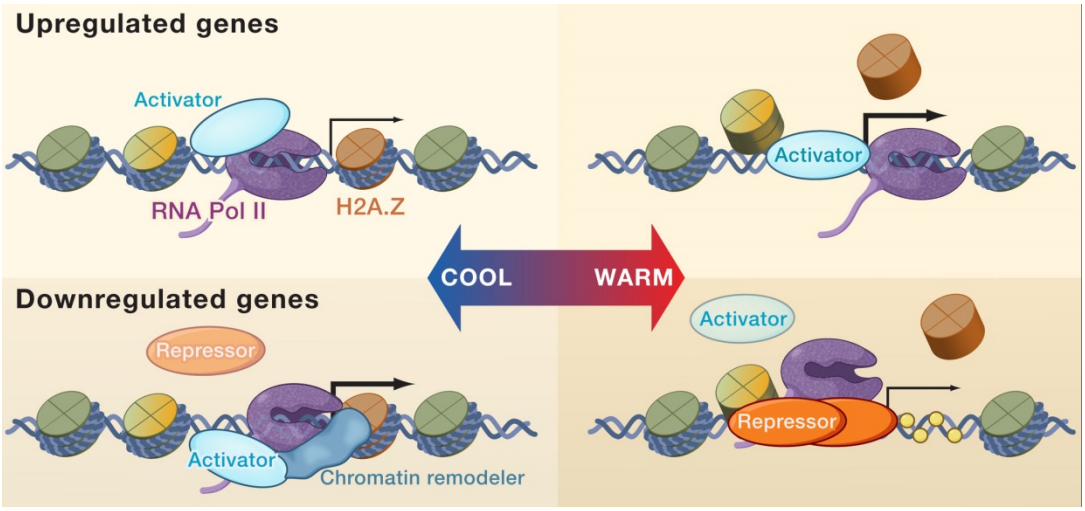

Figure 13. H2A.Z-Containing Nucleosomes Can Modulate Transcription in a Temperature-Dependent Manner. At lower temperature, H2A.Z nucleosomes have a high level of occupancy. H2A.Z nucleosomes may prevent transcription, either by acting as a physical block to the progression of RNA Pol II or by occluding gene-specific cis-elements from activating transcription factors. At higher temperature, H2A.Z nucleosome occupancy declines. This leads to increased expression of genes like HSP70, where transcription is limited by H2A.Z occupancy. For genes whose expression is decreased at higher temperature, this loss of H2A.Z may facilitate repressor binding (adapted from Franklin, 2010).

Objective and Organization of the thesis

Histone acetylation/deacetylation play important roles in a diverse range of developmental processes and stress-responsive pathways in plants. However, little is known regarding to the regulatory mechanism of HDACs themselves by environmental signals which may alter their function in regulation of gene expression, and HDACs functions in plant sensing envromental conditions such as redox stresses and warm ambient temperature. My thesis work focuses on the analysis of redox-regulated posttranslational modification and its consequences on HDA19 function in gene regulation and in SA-mediated stress response on one hand, and function of HDA9, HDA15, and HDA19 in plant resposnes to warm temperature and thermal-related genes expression, on the other hand.

The first part of RESULTS aims to analyze the regulatory mechanism of SA-mediated redox modification in HDA19 that control the expression of stress responsive genes. The key points of this part are: (1) the effects of SA treatment on the *HDA19* transcription and protein accumulation; (2) the SA-mediated redox modification of HDA19; (3) subcellular localization of HDA19 and the HDA19 nuclear accumulation upon SA treatment; (4) effects of HAD19 inactivition on redox-related gene expression.

The objective of the second part of the thesis is to investigate the biological and molecular functions of HDACs (HDA9, HDA15 and HDA19) in plant responses to warm temperature. To examine the biological function, the typical thermomorphogenesis phenotypes of *hda9*, *hda15* and *hda19* mutants were evaluated under different light and temperature conditions. For the molecular functions, temperatur-sensitive mark genes expression was validated by qRT-PCR in the mutants. Moreover, transcriptomes of *hda9*, *hda15* and *hda19* mutants grown under different temperature conditions were analyzed by RNA-Seq. Many differentailly expressed genes were identified between mutants and the wild type under both normal and warmer temperatures. Genes enriched in specific functional categories were identified by gene ontology analysis. The analysis revealed important but different roles of the HDACs in warm temperature resposnes.

Finally a conclusion and perspectives section is provided to discuss the main results and outline the whole work.

MATERIALS AND METHODS

2.1 Materials

2.1.1 Mutants and transgenic lines of plants

List of mutants and transgenic lines used in this study are described in **Table 3**.

Table 3 List of mutants and transgenic lines used in this study

Gene ID/Construct/Alias	Allele	Background	Mutagen	Original seed source ID
At4g38130, HDA19	*hda19*	Ws	TDI*	(Tian et al, 2003)
At4g38130, ***35S:HA-HDA19***		Ws	Transgene	This work
At4g38130, ***35S:GFP- HDA19***		Ws	Transgene	This work
At3g44680, HDA9	*hda9-1*	Col-0	TDI	SALK_007123
At3g44680, HDA9	*hda9-2*	Col-0	TDI	GABI_305G03
At3g44680, ***35S:HDA9-FLAG-HA***		Col-0	Transgene	This work
At3g1852, HDA15	*hda15-1*	Col-0	TDI	SALK_004027

Col-0: Columbia; * TDI: T-DNA Insertion; Ws: Wassilewskija

2.1.2 Oligonucleotide primers

Primers used in this study were obtained from Eurofins Genomics (Germany), and are listed in **Tables 4**. Primers used for molecular cloning were designed using the Primer 3 software at (http://www-genome.wi.mit.edu/cgi-bin/primer/primer3_www.cgi) and checked for secondary structures and melting temperature with PerlPrimer software.

Table 4 List of *HAD19* gene-specific primers used for cloning, mutation and genotyping of transgene

Cloning	**Forward primer sequence**	**Reverse primer sequence**
35S:HA-HDA19	AAAGTCGACGATACTGGCGG CAATTCGCTG	TTTGCGGCCGCGCTTATGTTTT AGGAGGAAACGC
35S:GFP-HDA19	AAAGTCGACATGGATACTGGC GGCAATTCGCTG	TTTGCGGCCGCGCTGTTTTAG GAGGAAACGC
Mutation	**Forward primer sequence**	**Reverse primer sequence**
HDA19-C137A	GCTTAACCACGGCCTCGCCGA TATTGCCATCAACTGGGC	GCCCAGTTGATGGCAATATCG GCGAGGCCGTGGTTAAGC
Genotyping for transgene	**Forward primer sequence**	**Reverse primer sequence**
35S:HA-HDA19	AAGCTTGCATGCCTGCAGGTCC CCAGA	TTTGCGGCCGCGCTTATGTT TTAGGAGGAAACGC
35S:GFP-HDA19	AAAGTCGACATGGATACTGG CGGCAATTCGCTG	GACTCTAGAGGATCCGACG ACATGAGT

2.1.3 Bacteria strains and plasmids

All bacteria and plasmids used in this study are listed in **Table 5** and **Table 6**.

Table 5 List of bacteria strains used in this study

Bacteria strain	Application
E. coli strain DH5α	Plasmids amplification
Agrobacterium tumefaciens GV3101	Genetic transformation

Table 6 List of plasmids used in this study

Name of plasmid	Selection in bacteria	Application
pENTR 1A	Kanamycin (50 μg/mL)	Complementary transgenic plants (Gateway entry donor)
pGWB5	Kanamycin (50 μg/mL)	Complementary transgenic plants (Gateway destination vector)
pGWB15	Kanamycin (50 μg/mL)	Complementary transgenic plants (Gateway destination vector)
pLA 1300	Kanamycin (50 μg/mL)	Complementary transgenic plants

2.1.4 Enzymes and antibodies

Enzymes and antibodies used in this study are listed in **Table 7 and 8**.

Table 7 Enzymes used for cloning, protein extraction and RT-qPCR

Enzyme	Providor
Sal I restriction enzyme	Promega, USA
Not I restriction enzyme	Promega, USA
Pfu DNA polymerase	Promega, USA
G2 fexi DNA polymerase	Promega, USA
Ribonuclease inhibitor	Promega, USA
Reverse transcriptase, RNase	Promega, USA
SYBR green I master	Roche, USA
Protease inhibitor (complete EDTA-free)	Roche, USA

Table 8 List of antibodies used in this study

Antibody	Origin	Provider	Cat. No	Application
Anti-H3 histone	Rabbit	Abcam	ab1791	Western Blot
Anti-H3ac	Rabbit	Millipore	06-599	Western Blot
Anti-H3K9ac	Rabbit	Millipore	07-352	Western Blot
Anti-H3K14ac	Rabbit	Millipore	07-352	Western Blot
Anti-HA	Rabbit	Sigma-Aldrich	H6908	Immunoprecipitation
Anti-HA (HRP)	Mouse	Sigma-Aldrich	H6533	Western Blot
Anti-GFP (HRP)	Goat	Abcam	ab6663	Western Blot

2.2 Methods

2.2.1 Growth conditions

2.2.1.1 Bacteria

Bacteria were grown either in Luria-Bertani (LB) liquid medium or on LB plates solidified with 0.8% agar that amended with an appropriate antibiotic. *E. coli* strains were grown at 37 °C and *Agrobacterium* GV3101 strains were grown at 30 °C. All antibiotics were prepared as 1000-fold concentrated solutions and kept at -20 °C.

2.2.1.2 Plants

The *Arabidopsis* seeds were surface-sterilized by treating with mixture liquid of 95% ethanol and 100% sodium hypochlorite solution (9:1) for 8 minutes, and washed with 95% ethanol for 3 times. Then seeds were sown on half Murashige and Skoog (1/2 MS) basal salt medium (Duchefa, Haarlem, The Netherlands). After stratification for 48 hours at 4 °C, seeds were transferred into a growth chamber containing incandescent light (100-200 μmol m-2 s-1), and grown for 10 days at 20 °C, under a 16/8 h or 8/16 light-dark cycle corresponding to long day (LD)- or short day (SD)-photoperiod conditions, respectively. Plants were either planted in the soil, or directly collected for RNA extraction or protein extraction.

2.2.2 Plants transformation

The transformation of *Arabidopsis* was performed by the floral dip technique as described (Bechtold and Pelletier, 1998). Briefly, Inflorescences of 3- or 4-week-old plants were dipped into *Agrobacterium tumefaciens* solution (OD600~0.8) with 5% sucrose and 0.05% Slivett L-77. To increase the infection efficiency, plants were alternately transformed twice a week and covered with a plastic wrap for 2 days to guarantee high humidity.

2.2.3 Plant treatments

2.2.3.1 SA/ GSNO treatment of *Arabidopsis* seedlings

Seedlings of 10-day-old *Arabidopsis* grown in 1/2 MS agar plates were treated with water or 0.5 mM sodium salicylate (Sigma-Aldrich; 31493) or 500 uM S-nitrosoglutathione (Sigma-Alorich; N4148) dissolved in water for different time points.

2.2.3.2 Warm temperature treatment and hypocotyl measurement

Upon germination at 20 °C, seedlings were either transferred to 27 °C or retained at 20 °C for 7 days unless otherwise specified. The light intensity of 200 μmol m-2 sec-1 was used to grow seedlings for all the experiments. Experiments were performed under LD, SD or dark conditions as specified. At least 20 seedlings were imaged and hypocotyl lengths were measured using NIH ImageJ software for each experiment with 3 times independent experiments repeat. The phenotypes of seedlings hypocotyl length were observed by stereomicroscope SV II (Zeiss) and the images were analyzed with the Adobe Photoshop Elements version 5.0 software (Adobe Systems).

2.2.4 Molecular methods

2.2.4.1 Methods for DNA

2.2.4.1.1 Plant DNA extraction with Edwards buffer

Frozen small leaves or entire seedlings in 8 attached 1.1 mL Microtubes containing beads were ground to fine powder with a grinding machine. 400 µL of Edwards Buffer (0.2 M Tris pH 8.0, 0.25 M NaCl, 0.025 M EDTA, 0.5% SDS) was added and mixed thoroughly to obtain a homogeneous solution. After 5 min of centrifugation at room temperature at 4000× g, the supernatant was collected into the clean Microtube.

DNA was precipitated by addition of 300 µL of isopropanol. After an additional centrifugation for 20 min, the supernatant was discarded and the pellet was washed with 1 mL of 70% Ethanol. The pellet was dried at 37 °C overnight, then re-suspended in 100 µL of sterile distilled water and stored at 4 °C until the analysis.

2.2.4.1.2 Molecular cloning

The whole *ATHDA19* cDNA sequences were amplified. Pfu High-Fidelity DNA Polymerase and specific primers **(Table 4)** were used to make restriction enzymes *Sal I* and *Not I* connect to *ATHDA19* cDNA N- and C-terminals. The PCR products were purified from agarose gel by using a gel extraction kit (Promega, A9281) according to manufacturer's recommendations. Purified PCR products were subcloned into entry donor plasmid pENTR 1A by restriction enzymes cohesive ends. The constructs were confirmed by sequencing. Then *ATHDA19* cDNA was cloned into gateway system destination plasmids pGWB5, pGWB15 and pDEST17 by LR recombination reaction (ThermoFisher; 1179100). The plasmids pGWB5, and pGWB15 were subsequently transformed into *c tumefaciens GV3101* for transgene work; the plasmid pDEST17

was subsequently transformed into BL21 for protein expression.

2.2.4.1.3 Site-directed mutagenesis

Mutants of HDA19 were generated using the QuikChange Site-Directed Mutagenesis kit (Agilent Technologies; Cat#210518). Cysteine 137 of HDA19 protein was mutated to alanine (C137A). The primers were showed in the **Table 4**.

2.2.4.2 Methods for RNA

2.2.4.2.1 RNA isolation and qRT-PCR

Total RNA was extracted from seedlings or rosette leaves using TRIzol Reagent according to manufactures recommendations (Invitrogen). For qRT-PCR analysis, 4 μg of total RNA was treated with DNase I (Promega) to eliminate any accidental DNA contaminations. The DNA-freed RNA was reverse-transcribed using Reverse Transcriptase RNase. Aliquots of the cDNA were used as template for qPCR with gene-specific primers and SYBR green master mix on a LightCycler 480 II Real time PCR cycler (Roche, USA). β-actin (FP: CGCTGACCGTATGAGCAAAGA; RP: GCAAGAATGGAACCACCGATC) was used as reference gene, and expression levels were expressed as relative to the control treatment. All samples were run with triplicate and results present are the mean (±s.d) of three biological replicates.

2.2.4.2.2 RNA-seq library preparation

Seedlings from wild-type (Col-0 and Ws) and mutants (*hda9-1, hda15-1 and hda19*) were pooled as two independent biological replicates in a transcriptomic analysis. High-quality total RNA was used for RNA-seq library construction. Briefly, 2 μg of total RNA was used for mRNA purification and cDNA synthesis. Then the mRNA was enriched by using oligo (dT) magnetic beads. Mixed with the fragmentation

buffer, the mRNA was broken into short fragments (about 200bp). Then the first strand of cDNA was synthesized by using random hexamer primer. Buffer, dNTPs, RNase H and DNA polymerase I were added to synthesize the second strand. The double-strand cDNA was purified with magnetic beads. End reparation and 3′-end single nucleotide A (adenine) addition was then performed. Finally, sequencing adaptors were ligated to the fragments. The fragments were enriched by PCR amplification. The library products were sequenced with the Illumina HiSeq 2 000 platform, and the library construction and sequencing were completed at BGI (Shenzhen, China).

2.2.5 Methods for proteins

2.2.5.1 Total protein extraction

Leaf tissues of 3-week-old *Arabidopsis* or 10-day-old entire seedlings (100-250 mg) were harvested in liquid nitrogen and ground to a fine powder. The samples then were transferred into cold 2 mL tubes on the ice, and added 1mL protein extraction buffer (0.12 M Tris pH 8.8, 0.25 M EDTA, 10% SDS, 50% glycerol) with protease inhibitor cocktail. The samples were vortexed for 15-30 seconds and placed on the ice for 5 minutes then centrifuged the suspension twice at 12000× g for 20 minutes at 4 °C. Protein concentration was analyzed by Qubit Protein assay kits (Thermofisher scientific; Q33211), according to manufacturer's recommendations.

2.2.5.2 Nuclear-Cytoplasmic fractionation

Leaf tissues of 3-week-old *Arabidopsis* or 10-day-old entire seedlings (100-250 mg) were harvested in liquid nitrogen and ground to a fine powder with 2 mL/g of lysis buffer (20 mM Tris-HCl pH 7.5, 20 mM KCl, 2 mM EDTA, 2.5 mM $MgCl_2$, 25% glycerol, 250 mM Suc, and 5 mM DTT) and protease inhibitor cocktail. The homogenate was filtered through a double layer of Miracloth. Then the flow-through

was spun at 1500× g for 10 minutes, and the supernatant, consisting of the cytoplasmic fraction, was centrifuged at 10,000× g for 10 minutes at 4 °C and collected. The pellet was washed four times with 5 mL of nuclear resuspension buffer NRBT (20 mM Tris-HCl pH 7.4, 25% glycerol, 2.5 mM $MgCl_2$, and 0.2% Triton X-100). Then the pellet was re-suspended with 500 mL of NRB2 (20 mM Tris-HCl pH 7.5, 0.25 M Suc, 10 mM $MgCl_2$, 0.5% Triton X-100, and 5 mM β-mercaptoethanol) supplemented with protease inhibitor cocktail and carefully overlaid on top of 500 mL NRB3 (20 mM Tris-HCl pH 7.5, 1.7 M Suc, 10 mM $MgCl_2$, 0.5% Triton X-100, and 5mM β-mercaptoethanol) supplemented with protease inhibitor cocktail. The mixture was centrifuged at 16,000× g for 45 minutes at 4 °C. The final nuclear pellet was re-suspended in 400 mL lysis buffer.

2.2.5.3 Histone extraction from *Arabidopsis*

Approximately 1 g of 10-day-old entire *Arabidopsis* seedlings was ground to a fine powder and homogenized in 8 mL histone extraction buffer (10 mM Tris-HCl pH 7.5, 2 mM EDTA, 0.25 M HCl, 5 mM β-mercaptoethanol, 0.2 mM phenylmethylsulfonyl fluoride, PMSF) on the ice for 15 min. After 12000× g centrifugation at 4 °C for 10 min, total histone proteins were incubated with concentrated Trichloroacetic acid (TCA) for 30 minutes on the ice in a 2 mL tube. The mixture then was precipitated from the supernatant by centrifugation at 17000× g for 30 minutes at 4 °C. After that, the supernatant was thrown and the histone pellets were washed once with acetone, air dried and then dissolved in distilled water with 4× Laemmli buffer and subjected to SDS-PAGE followed by Western blotting.

2.2.5.4 Protein separation on SDS-PAGE and Western-blotting

The protein samples were loaded on 4-12% SDS-polyacrylamide gels and were blotted using iBlot Gel Transfer Stacks (Invitrogen; Polyvinylidene difluoride membrane, IB401002; nitrocellulose membrane, IB301002). The transfer was left to occur at 15 V for 10 min on an iBlot Trans blot machine (Invitrogen). The membrane was subsequently blocked with 5% nonfat milk or 3% albumin from bovine serum in

Tris-buffered saline Tween-20 (TBST) or Phosphate Buffered Saline Tween-20 (PBST) at room temperature for 1h or 4 °C overnight. After that, the membrane was incubated with the desired primary antibody at room temperature for 1h or 4 °C for 3 h to overnight. Then the membrane was washed for 3 times with TBST or PBST and incubated with horseradish peroxidase labeled secondary antibodies for an additional 1h. For protein detection, the ECL Western blotting kit was used (Millpore; WBKL S0100) and documented on a ChemiDoc MP imaging system (Bio-Rad). As quality control for the fractionation, β-actin proteins were detected and used as cytoplasmic markers, and histone H3 was probed and used as nuclear markers.

2.2.5.5 Immunoprecipitation and S-Nitrosylation modification

10-day-old entire seedlings (500 mg) were ground to a fine powder in liquid nitrogen to extract total protein. 200-300 µg of total proteins were mixed with 2 µL of commercial antibodies and incubated at 4 °C on a roller for 2-3 h. 20 µl 50% Protein A agarose were centrifuged at 10000× g for 20 seconds at room temperature then washed 3 times with RIPA buffer (Sigma-Alorich; R0278). The mixture of the total proteins, antibodies was incubated with agarose beads on roller for 2 h at 4 °C. After spinning down to remove the supernatant, the pellets were washed 3 times with RIPA buffer. The complexes of protein A-antibody-protein were further checked for S-nitrosylation modification using Pierce S-nitrosylation western blot kit (Thermofisher scientific; 90105), according to manufactures recommendations. Briefly, S-nitrosylated protein samples are first reacted with methyl methanethiosulfonate to block free sulfhydryls. S-nitrosocysteines are selectively reduced with ascorbate for labeling with the iodoTMTzero reagent. Finally, the anti-TMT antibody is used for Western blot detection of the TMT-labeled proteins.

2.2.5.6 Protein Subcellular Localization

Pieces of leaves from one-week-old seedlings were sampled randomly and mounted in water. Imaging was performed using a LSM880 inverted confocal laser scanning microscope (Zeiss). For GFP-HDA19 fluorescence analysis, the 488-nm excitation

line of an argon ion laser was used, and images were analyzed with Zen software.

2.2.6 DAB staining

Leaves of 4-week-old *Arabidopsis* plants were pre-bolting and sampled into a 15 mL falcon tube. 5 mL 3,3′-diaminobenzidine (DAB) staining solution were added to the falcon tube to stain the leaves. The falcon tube was gently vacuumed for 5 minutes by three times to make DAB staining solution infiltrate the leaves. Then the falcon tube was covered with aluminum foil and shacked at 80-100 rpm for 6 h at room temperature. After that, boiling the leaves samples in bleaching solution (ethanol: acetic acid: glycerol as 3:1:1) at 90-95 °C in a water bath for 15 minutes and then replacing the bleaching solution with fresh bleaching solution. After standing for 30 minutes, samples were stored at 4 °C.

2.2.7 Cellular NAD/NADP and GSH/GSSG contents measurements

Antioxidants were analyzed as previously described (Queval and Noctor, 2007) with the following modifications: the final pH for the NAD/NADP sample was between 6-7 and between 5-6 for GSH/GSSG samples.

RESULTS

Functional analysis of HDACs in *Arabidopsis*

3.1 Salicylic Acid-mediated Redox Signaling Regulates HISTONE DEACETYLASE 19 S-Nitrosylation in *Arabidopsis*

Running title: Posttranslational modification of HDA19 by SA-mediated redox signaling

Salicylic Acid-mediated Redox Signaling Regulates HISTONE DEACETYLASE 19 S-Nitrosylation in *Arabidopsis*

3.1.1 Abstract

In plants, salicylic acid (SA)-triggered accumulation and release of reactive oxygen or nitrogen species (ROS/RNS) into the cell take place in response to a wide variety of adverse environmental conditions including salt, temperature, cold stresses, and pathogen attack, among others, which may have the potential to change the epigenetic mechanism of gene regulation. *Arabidopsis* histone deacetylase HDA19 displays a global transcriptional regulatory activity and is involved in SA-mediated defense responses. However, little is known about the mechanisms regulating the HDA19 function. Here, we show that SA-induced redox changes negatively regulate HDA19 nuclear accumulation through a reversible S-nitrosylation *in vivo*. Treatment with SA, as well as with the physiological nitric oxide donor, S-nitrosoglutathione, increases the abundance of several histone acetylation marks of HDA19 in *Arabidopsis* seedlings. Moreover, *hda19* mutant lines display increased ROS/RNS-related genes expression as well as nicotinamide adenine dinucleotide and glutathione levels. These findings suggest that SA affects histone acetylation by decreasing HDA19 nuclear accumulation, resulting in histone hyperacetylation. Thus, our results define a novel mechanism by which SA-triggered redox signal is transduced to epigenetic machinery through S-nitrosylation of HDA19 in plant response to environmental stress.

3.1.2 Introduction

Salicylic acid (SA) is one of the most versatile regulators and as a phytohormone plays crucial roles in many developmental pathways and stress responses (Boatwright and Pajerowska-Mukhtar, 2013; Liu et al., 2015b; Volt et al., 2009). The functions of SA in plant immunity have been extensively characterized and dissected using genetic and biochemical approaches. For instance, mutant plants that are impaired in the SA synthesis failed to express pathogenesis-related (*PR*) genes upon pathogen infection and the pathogen resistances of the mutant lines were severely compromised (Catinot et al., 2008; Strawn et al., 2007; Yang et al., 2015). In contrast, exogenously sourced SA to plants was shown to enhance plant resistance to pathogen infection (van Loon et al., 2006; Wang et al., 2007).

Recent evidences have shown that SA-mediated signaling pathways interact with redox signaling to respond environment stresses (Apel and Hirt, 2004; Spoel and Loake, 2011; Xia et al., 2015; Zottini et al., 2007). It has been shown that NONEXPRESSER OF PR GENES 1, a key player functioning downstream of SA in the plant defense response, alters its redox state in response to endogenous SA accumulation or exogenous SA treatment and translocates from the cytosol to nucleus to activate transcription of defense genes (Mou et al., 2003; Tada et al., 2008). Conversely, changes in cellular redox state also modulate SA synthesis, leading to expression of pathogen resistance genes (Volt et al., 2009; Yang et al., 2015; Zheng et al., 2015). Although the link between SA- and redox-mediated signaling pathways during stress response has been identified and, to some extent, characterized, details of the cross-talking mechanism remain largely unknown.

In plant, many processes are known to be regulated by histone acetylation, such as development and stress response (Ma et al., 2013; Servet et al., 2010; Shen et al., 2015). The homeostasis of histone acetylation is controlled by histone acetyltransferases (HATs) and histone deacetylases (HDACs) that catalyze respectively the transfer and removal of acetyl groups from acetyl-CoA to the N-terminal lysine (K) residues of histone proteins. A HDAC protein, HDA19, was shown to function as a global transcriptional regulator of gene expression involved in and plant developmental processes and responses to environmental changes (Chen et

al., 2015; Pi et al., 2015; Zhou et al., 2010). Knockout or overexpression of *HDA19* resulted in multiple defects in growth and development, such as abnormal leaves, delayed flowering, low fertility, etc. (Tian et al., 2003; Zhou et al., 2005). For instance, HDA19 is shown to repress seed maturation gene expression during germination (Zhou et al., 2013), which is likely to be achieved by functional interaction with another HDAC, HDA6 (Tanaka et al., 2008). Moreover, HDA19 is also shown to control the seed maturation program (Gao et al., 2015). Besides developmental functions, HDA19 has also been shown to be implicated in plant defense systems. It was first reported that HDA19 participates in the regulation of ethylene (ET)/jasmonic acid (JA) signaling pathways-mediated defense responses, because HDA19 overexpression up-regulates several ET/JA-induced genes and enhances resistance to a fungal pathogen (Zhou et al., 2005). In addition, it was proposed that HDA19 is involved in SA-mediated defense responses by positive regulation of PR1 expression (Choi et al., 2012). Although HDA19 plays important roles in several development and physiology processes in *Arabidopsis*, it remains unknown how HDA19 is involved in integration of stress responses and changes of cellular redox status to regulate gene expression and whether HDA19 activity itself is regulated the stress signals.

During recent years, several studies provided evidence of interaction between histone acetylation, stress responses, and redox homeostasis (Shen et al., 2016). The reactive oxygen or nitrogen species (ROS/RNS) generated during metabolism and stress responses perturb cellular redox states and cause oxidative modifications of enzymes and regulatory proteins affecting their activity, stability, or subcellular localization (Baxter et al., 2014; Vaahtera et al., 2014; Wrzaczek et al., 2013). Therefore, changes in the cellular redox balance may act as an effective signal that informs the cell of the prevailing environmental conditions to regulate the activity of chromatin modification activities such as histone acetyltransferase/deacetylases to reprogram gene expression for adaptation.. In mammalian, oxidative stress can modify the conformation of HDACs, resulting in altering their subcellular localization or reducing their catalytic activity. For instance, reduction of Cys-667/Cys-669 in HDAC4 inhibits its nuclear export, independently of its phosphorylation status (Ago et al., 2008). In addition, the oxidative stress induces modification at two conserved cysteine residues in HDAC1 and decreases its deacetylase activity (Doyle and Fitzpatrick, 2010). In *Arabidopsis*,

proteomics analysis indicated that HDA19 and HDA9 are among the identified redox-sensitive proteins. They are found to be oxidized upon SA treatment, which triggers the accumulation of ROS/RNS during early cellular responses (Liu et al., 2015a). It remains unknown whether the oxidation of HDA19 and HDA9 alters their activity or regulatory function. Recently, Mengel et al showed that S-nitrosoglutathione (GSNO), a physiological NO donor, as well as SA-mediated NO signaling inhibits overall HDAC activities *in vivo* (Mengel et al., 2017). Despite the role of HDA19 in SA-mediated gene expression has been demonstrated, whether HDA19 enzymatic activity and physiological function are regulated by SA-mediated oxidative modification is unclear.

In this work, we show that HDA19 is S-nitrosylated *in vitro* (in protein extracts) and *in vivo* (in seedling) upon GSNO or SA treatment, which negatively regulates the HDA19 nuclear accumulation, resulting in histone H3K9 and H3K14 hyperacetylation. We also found that HDA19 mutation led to up-regulation of several redox-related genes, as well as increased levels of the central metabolites that orchestrate plant cellular redox homeostasis. Our data indicate that the accumulation and function of HDA19 can be modulated by SA-mediated ROS/RNS signaling pathways, thus uncovering a molecular link between SA, redox modifications, and epigenetic regulation in stress responses in *Arabidopsis*.

3.1.3 Results

The effects of SA on the expression and accumulation of HDA19

As a first step to study whether SA modulated HDA19 function, we analyzed the expression of *HDA19* in 10-day-old *Arabidopsis* wild type seedlings by quantitative RT-PCR (qRT-PCR) after stimulation with 0.5 mM SA for different time periods. As shown in **Figure 1A**, the expression of *PR1* (AT2G14610), a well-known marker gene of the SA-mediated defense pathway (Choi et al., 2012), was significantly induced in SA-treated seedlings at 1 h and continued to increase gradually during the next 24 h, meaning that the SA treatment conditions (concentrations and durations) we used could trigger proper cellular stress responses. We found that the *HDA19* transcript was significantly elevated after SA treatment at 5h, but the induction was attenuated at 24 h, suggesting that SA may transiently induce *HDA19* transcription **(Figure 1B)**. In addition to HDA19, we also tested other two *Arabidopsis* RPD3/HDA1 HDACs (i.e., HDA6 and HDA9). Unlike *HDA19*, the expression of *HDA9* and *HDA6* was induced at 24 h after SA stimulation **(Figure 1C, 1D)**, suggested different timing of HDAC genes expression in responding to SA-signaling.

To test whether SA- affected HDA19 protein levels, we generated complementary transgenic plants by transforming *hda19* mutants (*athd1-1*, Wassilewskija [Ws] background) (Tian et al., 2003) with HDA19-HA or GFP-HDA19 fusion construct under the control of 35S promoter **(Figures S1A)**. Both transgenes fully rescued the developmental defects of *hda19* under the normal growth condition **(Figures S1B, and S2A-E)**. The *PR1* expression level in *hda19* was also restored to wild type level in the complementation plants **(Figure S3)**, suggesting that HDA19-HA or GFP fusion proteins were functional *in vivo*. The fusion proteins in the complementation plants were detected by western blotting using anti-HA or anti-GFP antibodies **(Figure S4)**. The analysis revealed that SA treatment significantly reduced the accumulation of -HDA19 fusion proteins after 2.5 h **(Figure 1E)**, indicating that the SA negatively regulates HDA19 accumulation. In transient increase of HDA19 transcripts at 5 h after treatment may be resulted from a feedback mechanism.

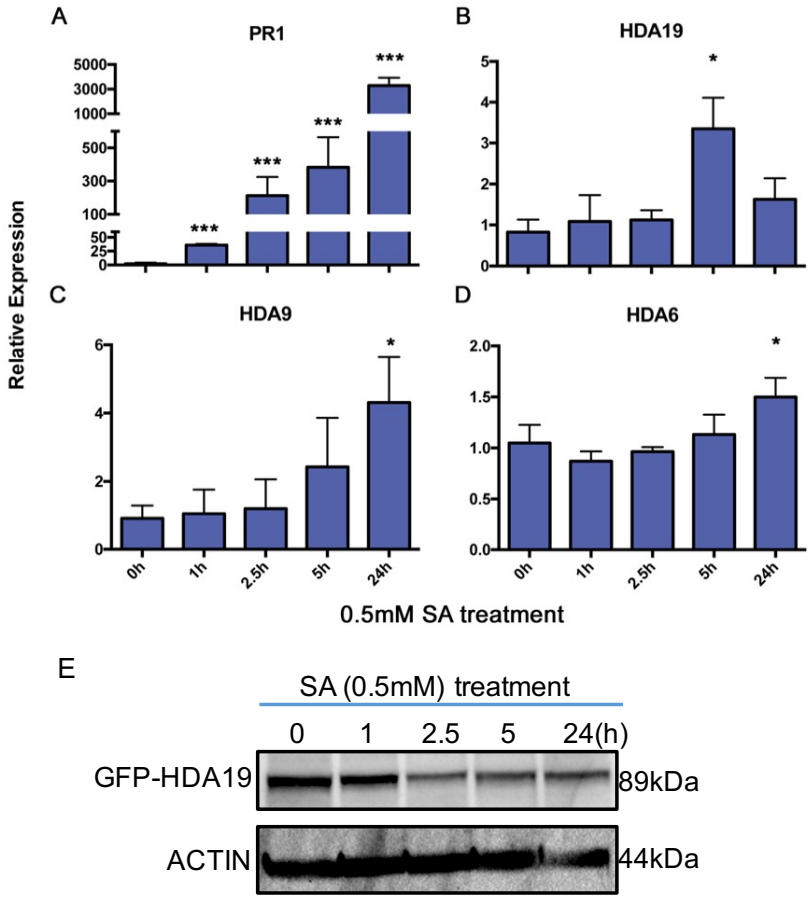

Figure 1. The effects of SA on the expression and accumulation of HDA19

(A-D) qPT-PCR analysis of *PR1*, as well as 3 Class I HDACs genes expression in 10-day-old wild type seedlings after SA treatment at different time points. β-actin was used as the internal standard. Three independent experiments were performed, each with two technical replicates, and generated similar results. Values are means ± SD (n=6). Primers were shown at **Supplemental Table 1**.

(E) Total proteins were extracted from 10-day-old *hda19*-35S:GFP-HDA19 complementation plant seedlings treated with 0.5 mM SA at different time points and probed against anti-GFP by western blot. Anti-actin is shown as a loading control. Experiments were repeated at least twice.

HDA19 is S-nitrosylated *in vivo* and *in vitro*

It has been shown that SA-mediated early burst of ROS/RNS, which occurs within several hours of stimulation, plays an important role in activation of the stress response through posttranslational mechanisms in *Arabidopsis* (Blanco et al., 2009; Overmyer et al., 2003). Among these posttranslational modifications, S-nitrosylation has been recognized as an important regulatory mechanism in SA-mediated signaling pathways controlling plant growth and development as well as stress responses (Lindermayr et al., 2010; Mou et al., 2003; Tada et al., 2008; Yun et al., 2016). To explore whether HDA19 was S-nitrosylated upon SA treatment, we employed the modified S-nitrosylation switch assay that uses a non-biological iodoTMT Reagent for labeling (Pierce S-nitrosylation Western blot kit, Thermo Fischer Scientific). Total HDA19-HA proteins from SA treated (3 h) 10-day-old transgene seedlings were purified using Protein-A agarose and then subjected to the S-nitrosylation biotin switch procedure. Subsequent western blot analysis with the iodoTMT antibody showed S-nitrosylation modifications of HDA19-HA protein in response to SA treatment **(Figures 2A, 2B)**. Congruent immunoblot analysis of HDA19-HA protein levels using anti-HA revealed a significant decrease in their relative abundance after 3 h SA treatment, compared to control (H_2O) **(Figure 2A)**, suggesting that S-nitrosylation may affect the HDA19 protein stability, which corresponded to the degradation of total HDA19-GFP proteins observed after 2.5 h SA treatment **(Figure 1E)**. We also investigated whether S-nitrosylation of HDA19 could occur *in vitro*. Purified HDA19-HA was incubated with a series of concentrations of GSNO, a predominant NO donor that directly regulates S-nitrosylation (Mur et al., 2013), and S-nitrosylation of HDA19-HA was monitored with the S-nitrosylation biotin switch assay. We found that HDA19-HA was S-nitrosylated in vitro **(Figure 2C)**. In addition, higher levels of GSNO produced higher S-nitrosylation of HDA19-HA, suggesting HDA19-HA was S-nitrosylated in a GSNO concentration-dependent fashion *in vitro*. Furthermore, the addition of dithiothreitol (DDT) significantly reduced the formation of S-nitrosylated HDA19-HA, suggesting a reversible thiol modification in HDA19 proteins. Together, these experiments revealed that HDA19 was S-nitrosylated *in vivo* and *in vitro*. To examine whether the degradation of HDA19 protein caused by SA treatment was due to the S-nitrosylation, total proteins were purified from 10-day-old transgene seedlings after 500 μM GSNO treatment for 3 hr, and analyzed by western

bolt using anti-HA. The results showed that the treatment resulted in a significantly reduction of HDA19-HA levels compared with untreated controls **(Figure 2D)**. Therefore, these date together suggested that S-nitrosylation affected the stability of HDA19 *in vivo*.

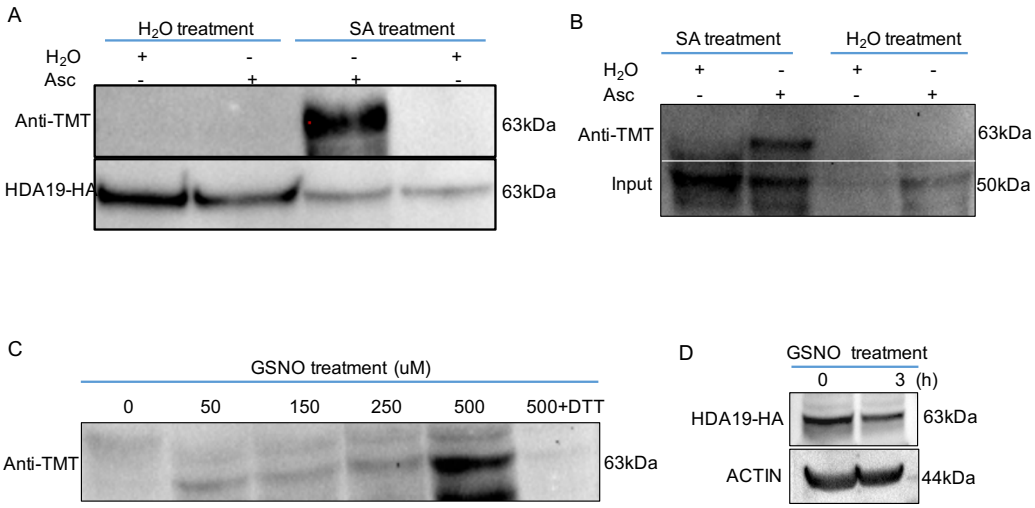

Figure 2. S-nitrosylation of HDA19

(A and B) Total protein extracts were made from 10-day-old of *hda19*-35S:HDA19-HA complementation plant seedlings treated with 0.5 mM SA after 3 hr. HDA19-HA proteins were immunoprecipitated using anti-HA and Protein Agarose-A then subjected to the S-nitrosylation biotin switch assay and subsequently immunoblotted with iodoTMT antibody. Two independent repeated experiments were shown.

(C) S-nitrosylation of purified HDA19-HA *in vitro*. Purified HDA19-HA proteins were incubated with the indicated concentrations of GSNO or GSNO plus dithiothreitol (DTT) and subsequently subjected to the S-nitrosylation biotin switch assay.

(D) Total proteins were extracted from 10-day-old *hda19*-35S:HDA19-HA complementation plant seedlings treated with 500 μM GSNO for 3 hr and probed against anti-HA by western blot. Anti-actin is shown as a loading control.

Experiments were repeated at least twice. Asc, ascorbate

HDA19 protein contains a number of Cys residues that might serve as sites for redox modification. In a proteomics analysis of redox-sensitive proteins in *Arabidopsis* cells, Liu et al. identified that HDA19 Cys-137 underwent reversible oxidative modifications in an early response to the treatment with SA and *flg22* (Liu et al., 2015a). To analyze the function of Cys-137, we generated transgenic plants carrying a *35S:HDA19-Cys137Ala-GFP/HA* transgene in the *hda19* mutant background. The point mutation construct could complement the *hda19* mutant phenotype **(Figures S5)**, suggesting that Cys137Ala mutation did not affect its function *in vivo*. By using the same analysis as described above, we found that the Cys137Ala mutation did not abolish *in vivo* S-nitrosylation of HDA19 induced by SA treatment, but displayed weaker anti-TMT labeling, compared with that of the wild type HDA19-HA protein **(Figures S6B, 2A)**. Interestingly, however, accumulation of the HDA19Cys137Ala-HA protein was higher after SA treatment for 3 hr **(Figures S6A)**, which was opposite to what observed in the wild type HDA19-HA. The observations suggest that Cys-137 is one of the S-nitrosylated sites in HDA19 induced by SA and S-nitrosylation may reduce accumulation of the protein.

SA-mediated S-nitrosylation negatively regulates the HDA19 nuclear accumulation

It has been well documented that the protein stability, subcellular localization as well as the biochemical activity can be modulated by S-nitrosylation (Astier et al., 2011; Hess et al., 2005). To gain mechanistic insights into SA-mediated HDA19 S-nitrosylation, we observed the subcellular localization of HDA19 in various intact living tissues of *HDA19-GFP* (in Ws background), as well as *HDA19Cys137Ala-GFP* transgenic complementation plants (in *hda19* mutant back ground) by performing confocal imaging analysis. The results showed that in the leaf and root tissues, HDA19-GFP existed in both nucleus and cytoplasm, but were absent in the nucleolus **(Figure 3)**. The GFP signal in the root tissue of Cys137Ala complementation plants displayed an indistinguishable pattern with the HDA19-GFP lines **(Figure S7)**,

suggesting that Cys137Ala point mutation did not influence the subcellular localization of HDA19 protein.

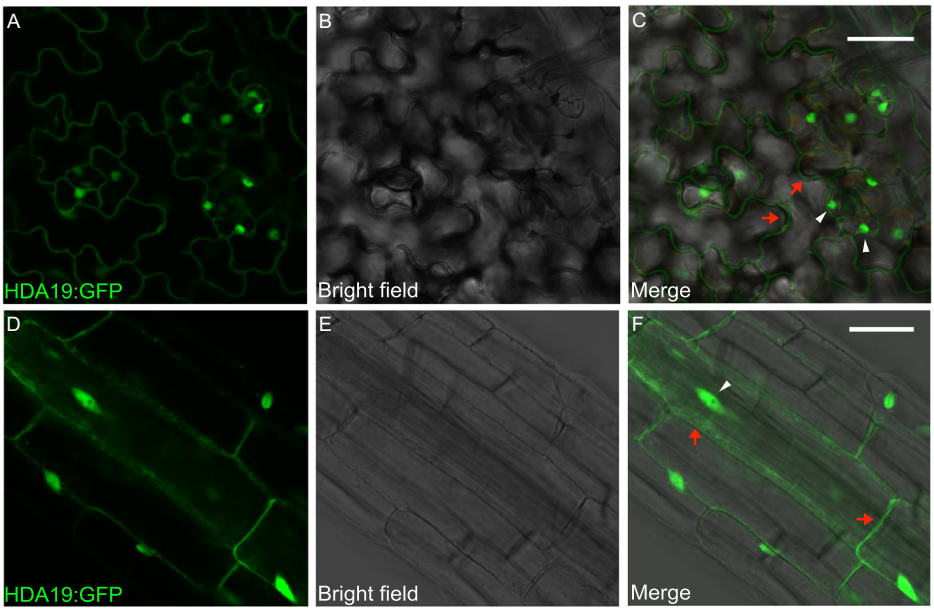

Figure 3. Subcellular localization of HDA19

7-day-old of *hda19*-35S:GFP-HDA19 seedlings were used to determine the subcellular localization of HDA19. HDA19 exhibited both nuclear and cytoplasmic localization, but not in the nucleolus regions (A-C, leaf tissues; D-F, root). Scale bars were calibrated to 20 mm.

The subcellular localization of HDA19-GFP in both nucleus and cytoplasm promoted us to examine whether SA affected HDA19 nuclear accumulation. In a nuclear-cytoplasmic fractionation assay followed by western blots, we found that the accumulation of HDA19-GFP in the nucleus was greatly reduced after SA treatment for 3 h, compared to the control plants (treated with H_2O), whereas the amount of protein was not significantly changed in the cytoplasm **(Figure 4)**, suggesting that S-nitrosylation affected the HDA19 protein accumulation mainly in the nucleus.

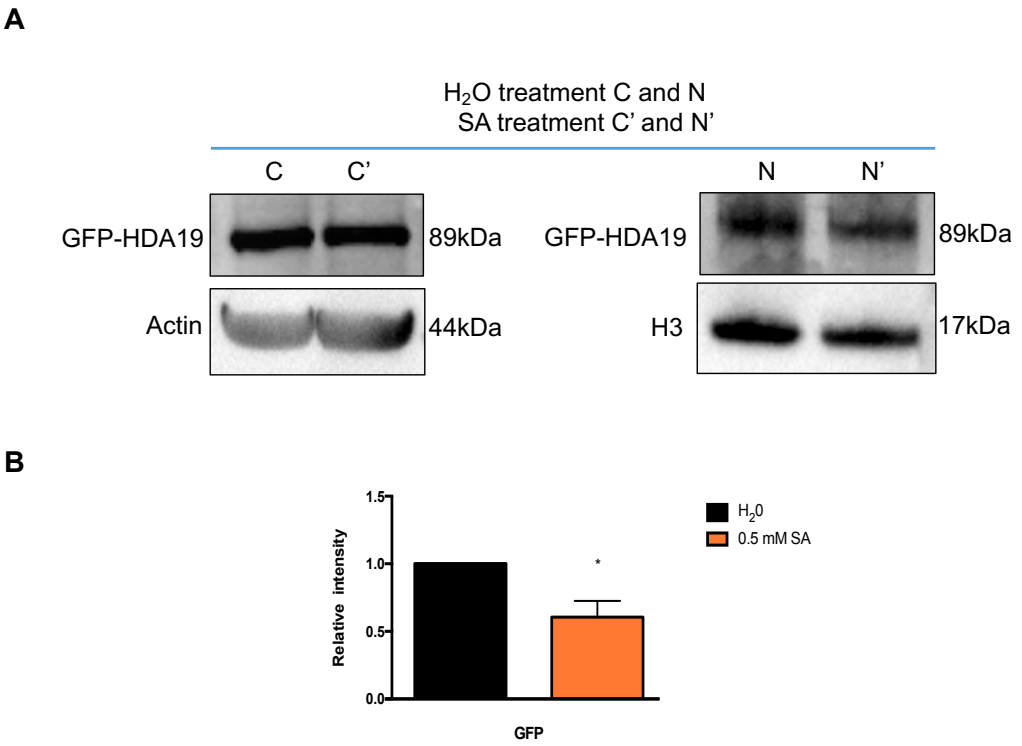

Figure 4. SA affects the HDA19 nuclear accumulation.

(A) Detection of HDA19-GFP protein in cytoplasmic (C), and nuclear (N) extracts in 10-old-day of *hda19*-35S:GFP-HDA19 seedlings after SA treatment for 3hr. Actin or Histone H3 is used as a control for nuclear or cytoplasmic proteins, respectively.

(B) Quantification of western-blot results. Signal intensities were measured using ImageJ software and normalized to the amount of loaded H3. Values are expressed as fold change over the control treatment. *, $P < 0.05$ by Student's *t* test.

Experiments were repeated at least twice.

SA-treatment induces H3 hyperacetylation *in vivo*

We next assessed a possible regulatory role of SA-mediated S-nitrosylation on the histone deacetylase activity of HDA19 *in vivo*. Ten-day old *Arabidopsis* seedlings were treated with 0.5 mM SA. After 3 h, histones were extracted and the acetylation level was determined by western blot. Previous studies revealed that HDA19 is critical for deacetylation of H3K9 (H3K9ac) and H3K14 (H3K14ac) in *vivo* (Tian and

Chen, 2001; Tian et al., 2003; Tian et al., 2005; Zhou et al., 2005) Accordingly, we found that SA treatment significantly increased the abundance of H3K9ac and H3K14ac, as well as total H3ac, in comparison with water treatment **(Figure 5)**, indicating that SA-triggered S-nitrosylation might inhibit the activity of HDA19 *in vivo*.

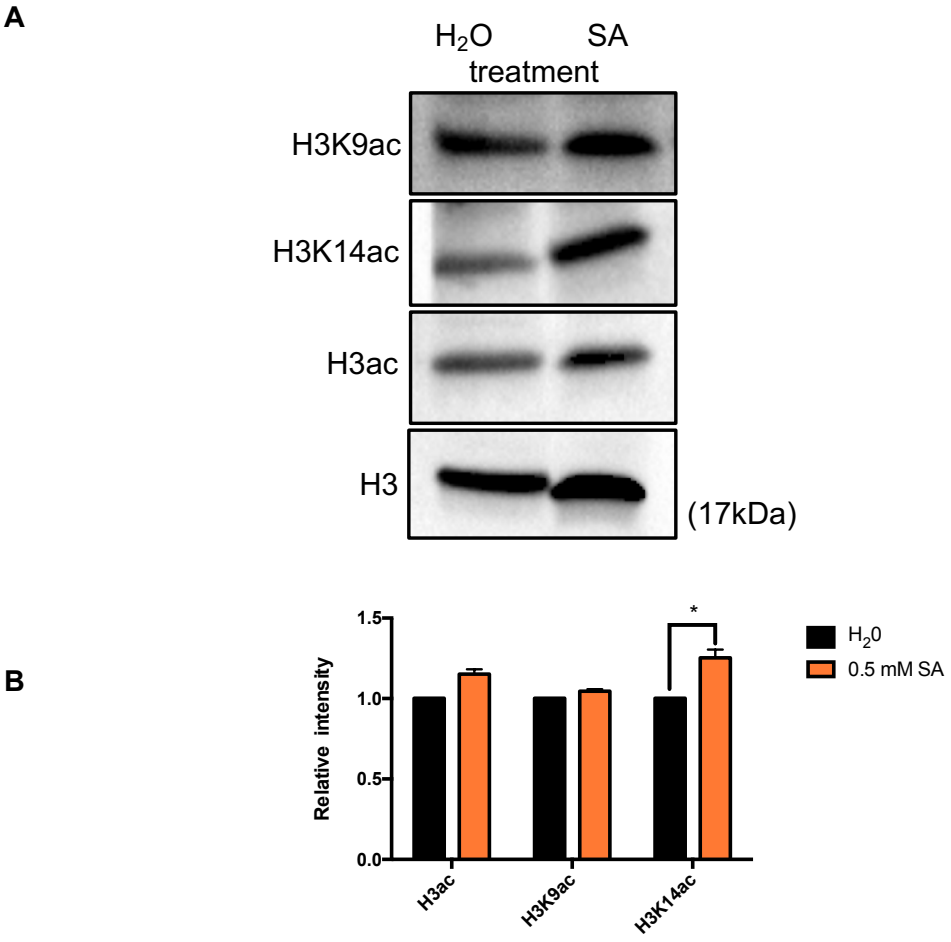

Figure 5. Histone acetylation in Arabidopsis seedlings after SA treatments

(A) Histones were extracted from 10-day-old of wild type seedlings treated with 0.5 mM SA or water (control) and probed against different histone acetylation marks by western blot.

(B) Quantification of western-blot results. Signal intensities were measured using ImageJ software and normalized to the amount of loaded H3. Values are expressed as fold change over the control treatment. *, $P < 0.05$ by Studen t's *t* test.

Experiments were repeated at least twice.

Loss of HDA19 causes increased expression of ROS/RNS related-genes

To gain more information about the involvement of redox signaling in HDA19 function, several types of redox-induced gene markers were analyzed by qRT-PCR in WT and *hda19* mutant seedlings: Glutathione S-transferase tau13 (*GSTU13*, AT1G27130), a $^1O_2$-induced marker gene (op den Camp et al., 2003; Ramel et al., 2012); Zinc Finger of Arabidopsis Thaliana12 (*ZAT12*, AT5G59820), an abiotic stress-induced transcription factor that plays a central role in H_2O_2-mediated signaling (Davletova et al., 2005; Le et al., 2016); Ferritin1 (*FER1*, AT5G01600), its expression was significantly induced by NO and whilst required for certain amount of glutathione (GSH) (Murgia et al., 2002; Touraine et al., 2012); Alternative Oxidase 1A (*AOX1A*, AT3G22370), which was shown to be induced in several ROS-producing conditions (Gadjev et al., 2006; Vaahtera et al., 2014), as well as a key regulator in JA/Et-mediated defense signaling, ethylene-responsive element-binding factors 5 (*ERF5*, AT5G47230) (Moffat et al., 2012; Son et al., 2012). As shown in **Figure 6**, qRT-PCR analysis revealed that the transcripts of all redox-related marker genes were strongly induced in *hda19* seedlings compared with those of the WT grown in normal conditions, suggesting that loss of HDA19 appears to activate various types of redox signaling under normal growth conditions.

H_2O_2 is the principal form of ROS and its accumulation links to a broad range of physiological processes, such as nutrient metabolism and pathogenic responses, etc. (Geigenberger and Fernie, 2014). We asked whether H_2O_2 perturbations contributed to the induction of redox-related genes expression in *hda19* mutant lines. Leaves of 4-week-old WT and *hda19* mutant plants were harvested and the accumulation of H_2O_2 in leaves was visualized by infiltrating leaves into diaminobenzidine (DAB) staining buffer (Fryer et al., 2003; Liu et al., 2007; Šnyrychová et al., 2008). Curiously, compared to what observed in WT leaves, the intensity of DAB staining in *hda19* leaves was not significantly changed **(Figure S8)**. Thus, the activation of redox gene expression in *hda19* mutant lines was not due to the H_2O_2-mediated oxidative burst.

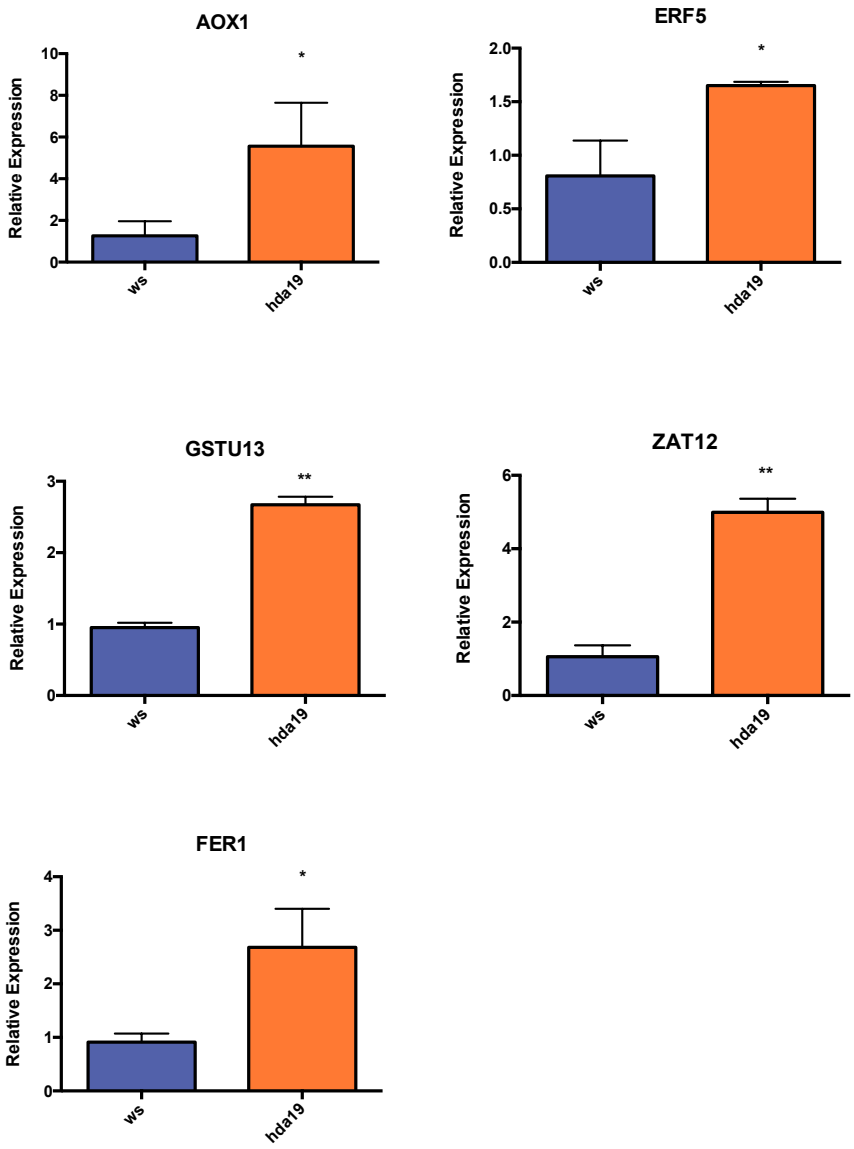

Figure 6. mRNA levels of redox-related genes in hda19 mutants

mRNA levels of serveral redox-related gene markers were determined by qPCR using gene-specific primer pairs. Values are expressed as mean ± SD of three independent experiments, normalized to β-actin mRNA level. Primers were shown at **Supplemental Table 1**.

NAD (H)/NADP (H) and glutathione content are increased in *hda19* mutants

To further test this whether the *hda19* mutation affected the cellular redox system, we measured the endogenous levels of nicotinamide adenine dinucleotide (phosphate) (NAD^+/$NADP^+$) and their corresponding reduced forms (NADH/NADPH) in the WT and *hda19* mutant plants, because NAD^+ and $NADP^+$ are traditionally known as the central metabolites orchestrating plant cellular redox homeostasis and their perturbations have been associated with stress redox signaling interactions (Apel and Hirt, 2004; Hashida et al., 2009). We found that there was a significant increase in the NAD^+ level in the mutants, but the level of NADH was not changed, this made the NADH/NAD ratio to decrease from 3.29% to 2.53% **(Figure 7A)**. The $NADP^+$ content only slightly increased in the mutant lines **(Figure 7B)**. Furthermore, as GSH status is not only considered to be a marker for changes in cellular redox state but also its system is potentially coupled with the NADPH oxidation (Geigenberger and Fernie, 2014; Mhamdi et al., 2012), we tested the contents of GSH and its oxidation form glutathione disulphide (GSSG) in the WT and *hda19* mutant plants. We observed that GSH levels were 1.45 fold higher in the *hda19* mutants compared to WT. But the GSSG levels did not change **(Figure 7C)**. Together, these data indicated that *hda19* mutation may not alter the overall cellular redox levels with increases of both NAD^+ (oxidized) and GSH (reduced) levels.

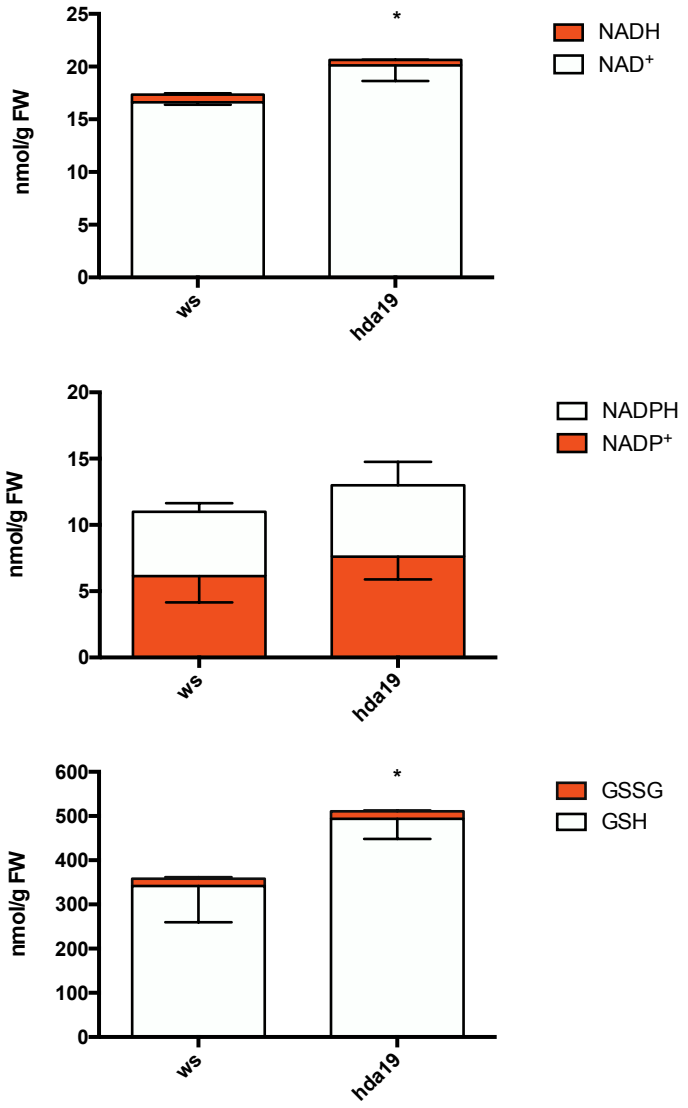

Figure 7. NAD (H)/NADP (H) and glutathione content are increased in hda19 mutants.

(A) NADH/NAD$^+$, (B) NADPH/NADP$^+$ and (C) GSSG/GSH ratios in the *hda19* mutant plants relative to wild type. Bars are means ± SD from three biological replicates and each replicate with three technical repeats. Significant differences are indicated (t test, n = 3), *P < 0.05.

3.1.4 Discussion

Chromatin regulators, especially histone modification enzymes such as HAT and HDAC, play an important role in gene expression involved in plant responses to the changing environment. It remains unclear how these regulators respond to environmental signals to target specific sets of genes in the genome. Genes encoding HAT and HDACs are generally expressed, although variations of their expression levels can be observed under different growth conditions. Interaction with DNA-binding transcription factors is suggested to target chromatin regulators to specific regions of the genome, while posttranslational modification is suggested to be a mechanism to regulate functions/or stability of chromatin regulators. However, there is little information regarding posttranslational modification of chromatin regulators in plants. In this work we provide evidence that the HDA19 protein is S-nitrosylated during SA-treatment. S-nitrolysation affects the stability of the HDA19 protein and its function in histone deacetylation and gene regulation. The result is consistent with recent data showing that stress-induced protein S-nitrosylation generally represses overall cellular HDAC function in plants (Mengel et al., 2017). Previous studies by mass spectrometry revealed that Cys-137 of HDA19 is oxidized during an early response to the treatment with SA and flg22 (Liu et al., 2015a). Our data showing that substitution of Cys137 to Ala could still complement the *hda19* mutant under normal conditions **(Figures S5)**, and did not abolish S-nitrosylation of HDA19 upon SA treatment **(Figures S6, 2A)** suggest that additional Cys residues of the proteins may be targeted for S-nitrosylation by SA-signaling and S-nitrosylation of more Cys residues may be necessary to alter HDA19 physiological function.

Similarly, it was shown that S-nitrosylation triggers degradation of the ABA-responsive transcription factor ABI5 to promote seed germination and seedling growth (Albertos et al., 2015). Conversely, nitric oxide produced during stresses triggers S-nitrolysation of Protein Arginine (R) Methyltransferase5 (PRMT5) and positively regulates the activity of enzyme (Hu et al., 2017). PRMT5 methylates arginine residues of both histone and non-histone proteins. Therefore, S-nitrosylation appears as a regulatory mechanism of stability or function of chromatin and transcriptional regulators in responding to environmental conditions.

Although HDA19 is shown to be involved in plant responses to stresses including those induced by JA and SA treatments, its precise role in stress responses remains unclear. Our data showing de-repression of SA-induced gene expression and increased expression of redox mark genes and higher NAD^+ and GSH levels indicate that HDA19 has a function to regulate redox gene expression and metabolite accumulation under normal conditions, which is likely to be achieved by repressing redox-related genes expression through histone deacetylation **(Figure 6)**. However, it is not excluded that HDA19 may also regulate lysine acetylation of enzymes involved in redox. Many metabolic enzymes have lysine acetylation that controls their activity. Moreover, HDA19 is detected in both nucleus and cytoplasm **(Figure 2)**, suggesting that this HDAC may deacetylate both histone and non-histone proteins including metabolic enzymes. This hypothesis waits to be checked by further experimentation. The subcellular localization patterns of HDA19 suggest that its function may be regulated by compartmentation. It appears that SA-signaling negatively regulates HDA19 stability and function, whereas HDA19 repressed SA-induced oxidative stress and gene expression under normal condition. Thus, HDA19 and SA-signaling seem to form a negative loop to regulate cellular redox states and gene expression to sense environmental conditions to control plant growth. Our results also suggest that HDA19 is not only a target of redox regulation but also controls cellular redox state to ensure plant growth under normal conditions. Redox states is tightly linked to plant energetic metabolism and histone acetylation enzymes play important roles to sense cellular energy status to regulate metabolism, growth and stress-responses (Shen et al., 2015, 2016). Whether HDA19 has a function to coordinate metabolism, stress-response and gene expression remains to be studied in the future.

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3.1.6 Supplemental data

3.1.6.1 Supplemental figures

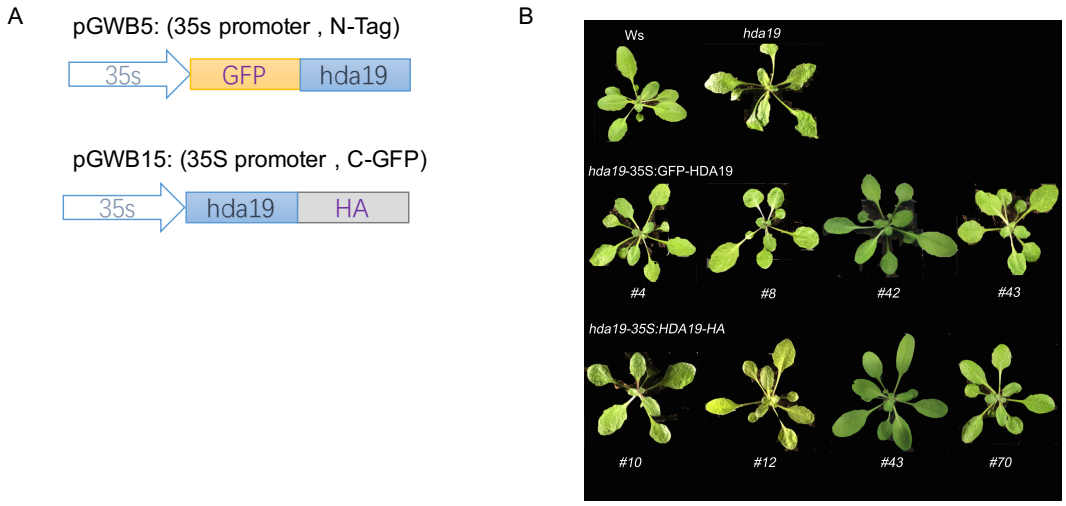

Figure S1. The generation of hda19 complementary transgenic plants

(A) Schematic representation of HDA19 fused GFP/HA protein.

(B) Photographs of 4-week-old plants. Altered leaf morphology such as leaf serration and twisted leaf orientation in *hda19* were rescued in *hda19* complementary transgenic plants.

Figure S2. Morphological phenotypes of hda19-35S:HDA19-HA complementary transgenic plants

(A) Transgenic plants along with corresponding *hda19* mutants and wild type grown in LD, respectively.

(B–E) The aberrant cotyledons, abnormal petals and reduced fertility of *hda19* were rescued in HDA19-HA.

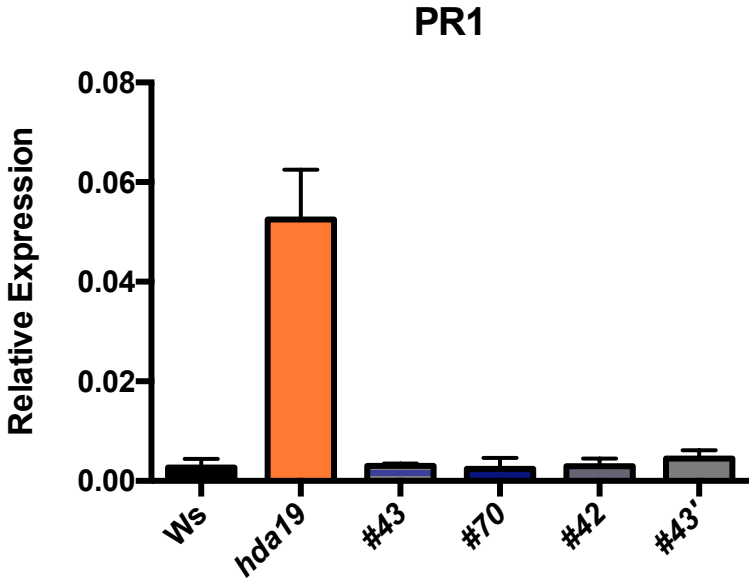

Figure S3. Expression of PR1 in wild type, hda19 and hda19 complementary transgenic plants

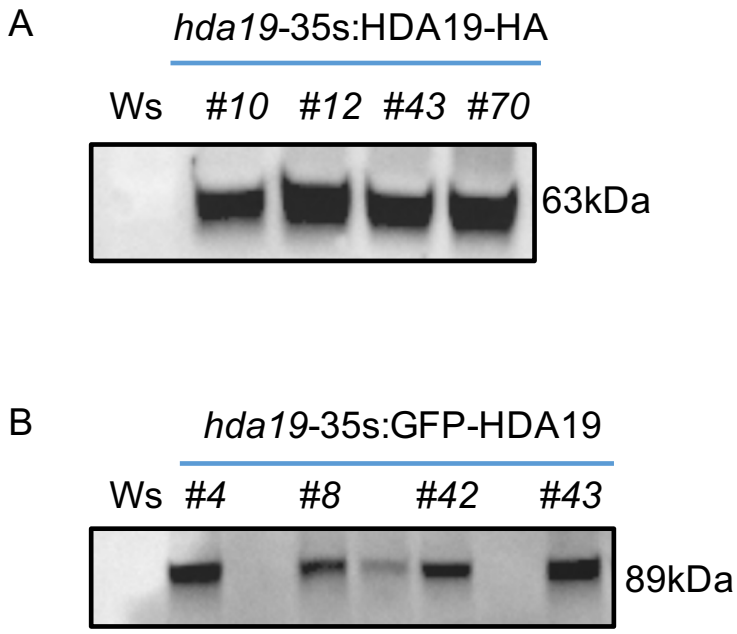

Figure S4. Western bolt analyses of the expression of epitope-tagged proteins HDA19-HA/GFP in hda19 complementary transgenic plants

Figure S5. Photographs of 4-week-old plants grown

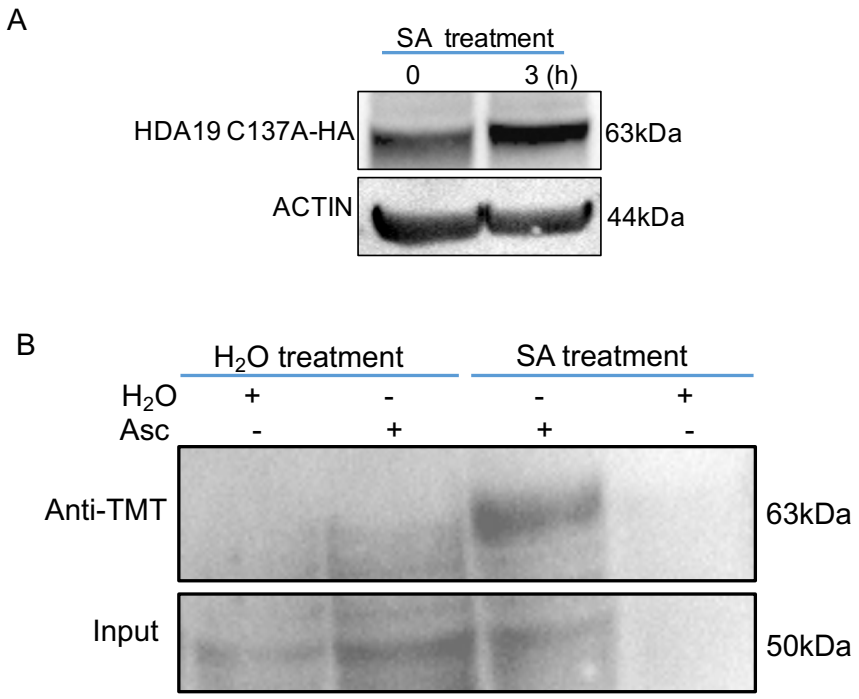

Figure S6. S-nitrosylation of HDA19Cys137Ala

(A) Total proteins were extracted from 10-day-old *hda19*-35S:HDA19Cys137Ala-HA transgenic plant seedlings treated with 0.5 mM SA for 3 hr and probed against anti-HA by western blot. Anti-actin is shown as a loading control.

(B) Total protein extracts were made from 10-day-old of *hda19*-35S:HDA19Cys137Ala-HA transgenic plant seedlings treated with 0.5 mM SA after 3 hr. HDA19Cys137Ala-HA proteins were immunoprecipitated using anti-HA and Protein Agarose-A then subjected to the S-nitrosylation biotin switch assay and subsequently immunoblotted with iodoTMT antibody.

Experiments were repeated at least twice.

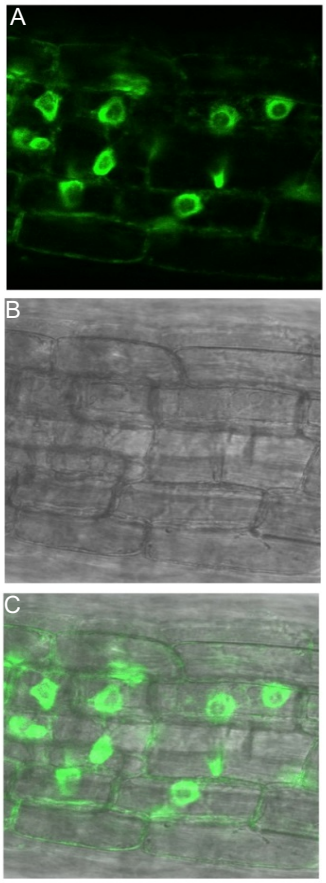

Figure S7. Subcellular localization of HDA19Cys137Ala-GFP

(A-C, root) 7-day-old of *hda19*-35S:HDA19Cys137Ala-GFP seedlings were used to determine the subcellular localization of HDA19Cys137Ala-GFP. HDA19Cys137Ala exhibited both nuclear and cytoplasmic localization.

Figure S8. Histochemical detection of H_2O_2 in leaves

Representative leaves of 4-week-old of wild type and *hda19* mutant plants stained with DAB.

3.1.6.2 Supplemental table

Table S1. Sequences of the primers used in this study

RT-qPCR	Forward primer sequence	Reverse primer sequence
ERF5	TTATGTGACTGGGATTTAACGGG	TCAAACAACGGTCAACTGGG
GSTU13	CCATCGGCTACCTTGACATT	TGTCTCTTGCCGGAGAAACT
ZAT12	TGTCCCATATGTGGAGTGGA	ATTGTCCACCATCCCTAGACT
AOX1	TGCCTTTTTCGCTGATTACG	CAAAAACAGCCATGACTCTCG
FER1	CGTTCACAAAGTGGCCTCAG	CGTTCACAAAGTGGCCTCAG
PR1	GCCGTGAACATGTGGGTTAG	GGCACATCCGAGTCTCACTG

3.2 Function of *Arabidopsis* HISTONE DEACETYLASES HDA9, HDA15, and HDA19 in plant responses to warm temperature

Running title: Function of histone deacetylases HDA9, HDA15 and HDA19 in thermomorphogenesis

Function of *Arabidopsis* HISTONE DEACETYLASES HDA9, HDA15, and HDA19 in plant responses to warm temperature

3.2.1 Abstract

Plants can respond to high temperatures through different signaling pathways. Although it has been well established on how plants cope with heat stress, the thermosensory mechanisms responding to increased ambient temperatures remains poorly understood. In this report, we show that the *Arabidopsis* RPD3-type histone deacetylases (HDA9, HDA15, and HDA19) are involved in modulating plant adaptation to higher ambient temperatures in *Arabidopsis*. Mutation of HDA15 displayed a warm temperature-induced phenotype under normal temperature, whereas mutation of HDA9 and HDA19 induced opposite effects showing a warm-temperature-insensitive phenotype. Gene expression and RNA sequencing analysis revealed that HDA15 mutation led to up-regulation of many genes that were involved in primary and cellular metabolic processes when the seedlings were transferred from 20 °C to 27 °C for 4 hr. On the other hand, *hda19* mutation resulted in up-regulation of genes mainly involved in stress-responses at both normal (20 °C) and warmer (27 °C) temperatures. However, up-regulated genes in *hda9-1* mutants seemed not to be enriched for any particular gene functional category. Likely, HDA9 and HDA19 positively regulate thermosensory elongation through distinct mechanisms. Our study suggested that histone acetylation dynamics regulated by HDA9, HDA15, and HDA19 plays an important role for plant adaptation to warm temperature, which involves distinct regulatory pathways of gene expression.

3.2.2 Introduction

Environmental cues, in particular temperature, affect plant growth and development and have dramatic effects on plant architecture and biomass throughout the entire lifecycle (Atkin et al., 2006; McClung et al., 2016; Patel and Franklin, 2014). In turn, plants have evolved sophisticated regulatory mechanisms to adapt quickly to their unfavorable surrounding environment (Ikeuchi et al., 2013; Samach and Wigge, 2005). Although a large body of literature is available on how plants cope with heat stress (Bäurle, 2016; Mittler et al., 2012; Liu et al., 2015), little is known about how plants respond to moderately increased ambient temperatures which becomes a serious concerns for ecology and agriculture, due to global warming occurred during the last century.

Plants have the potential to acclimate to higher ambient temperatures by inducing adaptive elongation growth of stems and leaves to enhance cooling capacity and avoid heat flux. This acclimation process is called thermomorphogenesis and is critical for plant fitness and yield. Although the regulation of thermomorphogenesis under warm ambient temperatures is a fundamental activity for plants, the molecular mechanism underlying this process is poorly understood. Only recently, the bHLH transcriptional regulator PHYTOCHROME INTERACTING FACTOR 4 (PIF4) was put forward as a central signaling hub for the integration in thermomorphogenesis signaling (Koini et al., 2009; Kumar et al., 2012; Leivar and Monte, 2014; Leivar and Quail, 2011; Paik et al., 2017; Proveniers and van Zanten, 2013). *pif4* mutants were unable to increase hypocotyl length at warmer temperatures (Koini et al., 2009). Moreover, PIF4 stimulated the production of the phytohormone auxin by directly binding to the promoter regions of auxin biosynthesis genes, which subsequently triggers elongation growth at warmer temperature conditions (Franklin et al., 2011; Sun et al., 2012). PIF4 was also found to be involved in brassinosteroid (BR)-mediated warm temperature-induced hypocotyl elongation process (Oh et al., 2012). Recent evidences revealed that light and temperature signals cross-communicate in determining adaptive growth responses in a PIF4-dependent manner. For instance, Gangappa and Kumar demonstrated that the well-described DET1-COP1-HY5 photomorphogenic pathway is required for upstream for induction of PIF4 under high temperature (Gangappa and Kumar, 2017). Moreover, thermosensory growth and architecture

adaptations were also shown to directly link to suppression of plant defenses at elevated temperature (Gangappa et al., 2017), revealing another physiological link between thermomorphogenesis and immunity in plants.

The regulation of gene transcription involves transcription cofactors (co-activator or co-repressor) capable of transducing signals from enhancer-bound specific transcription factors to the RNA polymerase initiation complex (Bertrand et al., 2005). *In vitro* studies have shown that transcription cofactors are usually associated with chromatin remodeling and modification activities such as replacement of canonical histones with specialized variants and ATP-dependent chromatin remodeling (Ho and Crabtree, 2010; To and Kim, 2014). Recently, there are growing evidences showed that epigenetic mechanisms are involved in regulating the plant thermomorphogenesis to influence the plant development under high ambient temperatures. Kumar and Wigge found that histone H2A variant, H2A.Z, is evicted from chromatin at transcriptonal start-sites at elevated temperatures. Thereby it contributes to thermomorphogenesis by allowing the binding of transcriptional regulators and the transcriptional machinery to temperature-regulated genes (Kumar and Wigge, 2010). In addition, Zha et al. demonstrated that PICKLE (PKL), an ATP-dependent chromatin-remodeling factor, controls thermosensory hypocotyl growth of *Arabidopsis* (Zha et al., 2017). PKL loss-of-function resulted in reduced sensitivity in hypocotyl elongation to warm temperature and PKL affected the level of trimethylation of histone H3 Lys 27 associated with INDOLE-3-ACETIC ACID INDUCIBLE 19 (IAA19) and IAA29 and regulates their expression. Although there are many lines of evidence indicating the significance of chromatin remodeling and modification in thermomorphogenesis of *Arabidopsis*, the molecular mechanisms of thermomorphogenic signaling pathway remain largely unknown.

Epigenetic modification of the structural proteins in chromatin alters the local chromatin structure, in particular chemical modifications of histone proteins by acetylation affect gene expression (Eberharter and Becker, 2005). The homeostasis of histone acetylation is controlled by histone acetyltransferases (HATs) and histone deacetylases (HDACs). HDACs catalyze the removal of acetyl groups from acetylated lysine (K) residues in histone proteins. A total of 16 *Arabidopsis* HDAC genes have been identified, of which 10 belong to the RPD3/HDA1 superfamily, 4 to the HD2

family and 2 to the SIR2 family (Pandey et al., 2002). Genetic ablation of the HDAC function has shown that HDACs play diverse and important roles in many aspects of development and physiology in *Arabidopsis*. We have previously shown that an *Arabidopsis* Class I RPD3/HDA1 HDACs, HDA19, is required for normal photomorphogenesis and light-activated gene transcription (Benhamed et al., 2006) and is also involved in defense responses (Choi et al., 2012; Zhou et al., 2005). We have shown that HDA9, belonging also to the RPD3/HDA1 group, regulates flowering time by deacetylating and repressing the expression of AGL19 gene (Kim et al., 2013), and function as a negative regulator of stress-responsive genes (Zheng et al., 2016). Moreover, another member of the Class II RPD3/HDA1 HDACs, HDA15, was shown as a key regulator of the light-controlled hypocotyl elongation via modulating histone acetylation under various light conditions (Liu et al., 2013; Tang et al., 2017). In this work, we show that HDA19, HDA9, and HDA15 are involved in thermomorphogenesis in *Arabidopsis*. Loss of function of HDA15 displayed constitutive hypocotyl elongation under normal ambient temperature condition, whereas mutation of HDA9 or HDA19 failed to respond to elevated temperature, resulting in shorter hypocotyls than that of wild type. Genetic and molecular analyses further suggest that HDA9 and HDA19 have a distinct function in the regulation of thermosensory responses. The results suggest that although belonging to the same group, different HDACs play distinct roles in thermomorphogenesis and provide new insights into plant integration of environmental signaling through epigenetic machinery.

3.2.3 Results

Different photoperiod affects thermosensory hypocotyl growth in *Arabidopsis*.

Light and temperature patterns being the most dynamic parameters are often correlated under normal plant growth environment. It is well known that both light and temperature strongly influence seedling hypocotyl elongation, a key trait of great adaptive significance (Arsovski et al., 2012; Penfield, 2008). Despite such functional relevance, the mechanism of simultaneously sensing light and temperature signals by plants has not been fully elucidated. To understand how the changes in these two environmental cues mutually influence plant growth, we studied seedling hypocotyl elongation in two genetic backgrounds of *Arabidopsis* (Columbia [Col-0] and Wassilewskija [Ws]) under varying photoperiod-temperature conditions. Wild type seeds were germinated under continuous white light at 20 °C. At day 2 after germination, seedlings were transferred to 27 °C or kept at 20 °C for additional 7 days. Hypocotyl lengths of seedlings were measured and statistically analyzed (see Methods) to determine significant differences. We found that both Col-0 and Ws seedlings grown at 27 °C displayed an elongated hypocotyl phenotype than that of those grown at 20 °C under all tested day-length conditions, including constant dark conditions **(Figures 1, S1)**, suggesting that the warm temperature-induced hypocotyl elongation can be separated from light response in *Arabidopsis*. However, we observed that the photoperiod did influence the extent of thermosensory growth. Seedlings grown under constant dark conditions exhibited largest elongated hypocotyls regardless of the temperature exposure, but lowest relative growth rate (27 °C versus 20 °C) than that grown either under long-day (LD, 16 hr light/8 hr dark) or short-day (SD, 8 hr light/16 hr dark) conditions **(Figures S1A, 1C)**. In contrary, SD condition induced robust temperature-responsive hypocotyl elongation of seedlings compared to LD condition where hypocotyls were shorter and showed only slight response to elevated temperature **(Figure 1)**, suggesting that the effect of warm temperatures on the induction of hypocotyl elongation is influenced by day lengths. *Arabidopsis* seedlings showed stronger responses to warm temperature under SD condition.

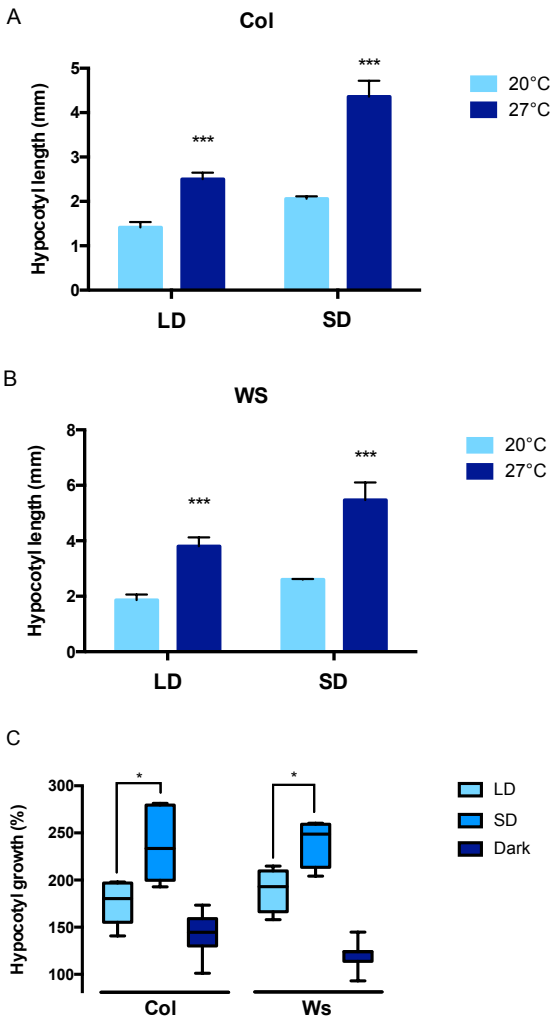

Figure 1. Thermosensory hypocotyl elongation response is influenced by photoperiod.

(A and B) Hypocotyl length of 10-day-old Col-0 and Ws seedlings grown in long-day (LD) and short-day (SD) photoperiod at 20 °C and 27 °C were measured (mean ± SD; n=60). ***P <0.001 (Student's t test) significantly different from Col-0 in corresponding photoperiod conditions or between different temperature conditions.

(C) Relative growth rate at 27 °C versus 20 °C of Col-0 and Ws seedlings grown in different photoperiod conditions. See also **Figure S1**.

HDA9, HDA15 and HDA19 mutants have distinct sensitivities to warm temperature-induced hypocotyl elongation

To examine whether HDAC genes are involved in temperature-regulated plant developmental processes, we used previously characterized multiple mutant alleles of HDA9 (*hda9-1* and *hda9-2*), HDA15 (*hda15-1*) (all in a Col-0 background), and HDA19 (*hda19*) (in Ws background) (Kim et al., 2013; Liu et al., 2013; Tian et al., 2003). In the dark, hypocotyl lengths of the different genotypes were similar, except *hda15-1*, which displayed longer hypocotyls than Col-0 seedlings at 27 °C **(Figure S1B)**, suggesting a negative role of HDA15 in warm temperature-induced hypocotyl elongation in the dark. In addition to the hypersensitivity to elevated temperature, *hda15-1* seedlings also showed a temperature-hypersensitive phenotype with longer hypocotyls compared with those of Col-0 plants under both SD and LD conditions **(Figures 2A, S2A)**. By contrast, hypocotyls of *hda9-1*, *hda9-2* and *hda19* seedlings were shorter than wild type hypocotyls at 27 °C, showing a reduced thermomorphogenic response **(Figures 2B, 2C, S2B, S2C)**. The abnormal phenotypes for all the HDACs mutants were most pronounced when grown under SD conditions, which is consistent with the above data with wild types seedlings **(Figure 3)**. Additionally, complementation of the *hda9-2* or *hda19* mutations with the HDA9 or HDA19 cDNA restored a normal-hypocotyl phenotype under warm temperature condition **(Figures 2B, 2C, S2C)**, further confirming the positive role of HDA9 and HDA19 in temperature-induced elongation growth. Furthermore, *hda9-1 hda15-1* double mutants were made using *hda9-1* pollen to fertilize *hda15-1*. The hypocotyl length of the double mutant was intermediate to that of *hda9-1* and *hda15-1* single mutants, but still lower than Col-0 seedlings at 27 °C **(Figures 2D, 3, S1, S2A)**, suggesting that HDA9 and HDA15 may play an antagonistic role in regulation of warm temperature-induced hypocotyl elongation. Taken together, these data suggested that different HDACs have distinct functions in thermosensory hypocotyl growth.

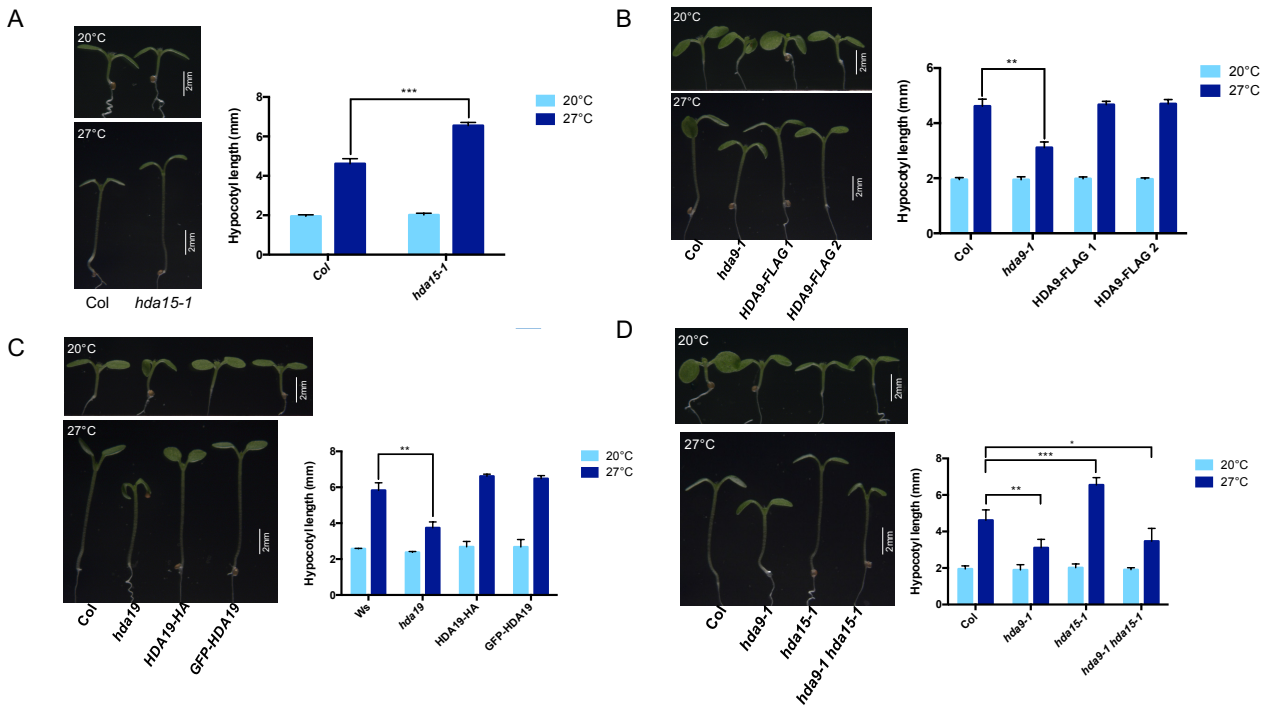

Figure 2. Elongation of hypocotyl in different mutant lines under SD condition

Representative image of seedling hypocotyl elongation in 10-day-old *hda15-1* (A), *hda9-1* and *hda9-2*-FLAG complementation seedlings (B), as well as *hda19* and *hda19*-HA/GFP complementation seedlings (C) along with wild type (Col-0 and Ws) grown at constant 20 °C and 27 °C under SD photoperiod (mean ± SD; n=60). **P <0.01, ***P <0.001 (Student's t test) significantly different from Col-0 in corresponding temperature conditions. Bar = 2 mm. See also **Figure S2**.

(D) Quantification of hypocotyl lengths of 10-day-old Col-0, *hda9-1*, *hda15-1* and *hda9-1hda15-1* seedlings at constant 20 °C and 27 °C under SD photoperiod conditions.

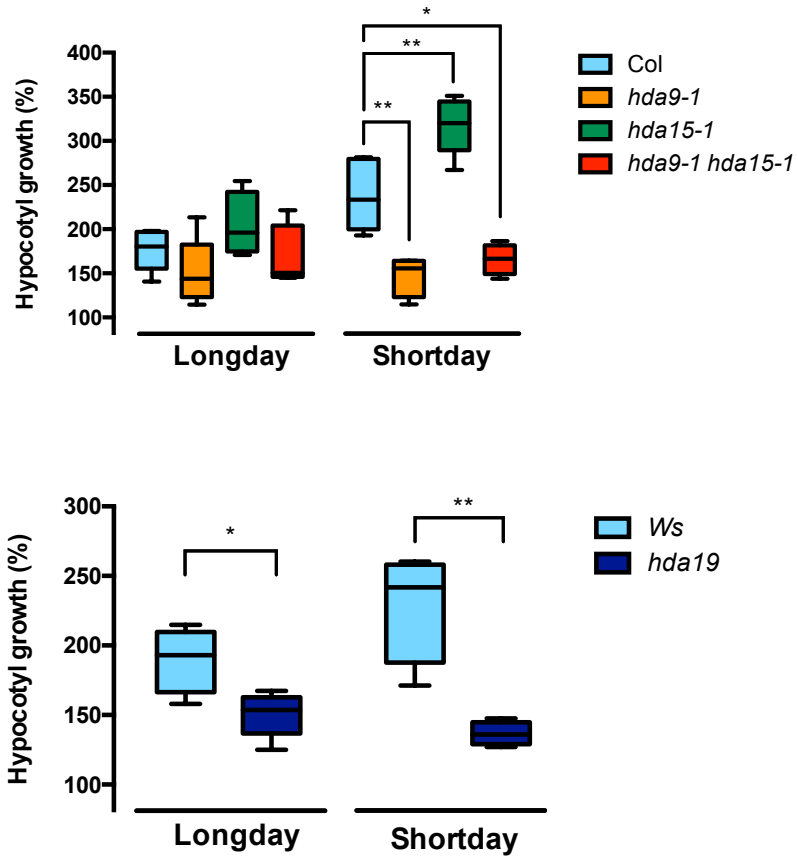

Figure 3. Relative growth rates at 27 °C versus 20 °C between different HDACs mutants in corresponding photoperiod conditions

HDA9, HDA15, and HDA19 have distinct effects on HY5-PIF4 mediated gene expression

To understand the molecular regulatory mechanism of HDACs in thermomorphogenesis, the well-described HY5-PIF4 mediated light/temperature-inducible gene expression in *hda9-1*, *hda15-1* and *hda19* mutants was analyzed by quantitative RT-PCR (qRT-PCR). Total RNA isolated from SD-grown 10-day-old seedlings that were shifted from 20 °C to 27 °C for 4 hr was analyzed with primers corresponding to the *PIF4* and its downstream target genes *YUC8* and *IAA29*, as well as its repressor *HY5*. As shown in **Figures 4 and 5**, all

tested light/temperature-induced genes displayed fluctuation depending on the circadian clock at 20 °C, which is consistent with the previous data (Legris et al., 2017). Moreover, we observed similar levels of temperature-induced expression as well as basal expression of all four genes between Col-0 and *hda15-1* mutants **(Figure 4)**, suggesting that HDA15 may not regulate HY5-PIF4 mediated signaling pathway in response to elevated ambient temperatures. By contrast, although the induction of *PIF4* transcript was not significantly modified in *hda9-1* mutants compared to wild type at 27 °C, the decreases of *IAA29* and *YUC8* transcripts were observed in *hda9-1* **(Figure 4)**, suggesting that HDA9 may act downstream of PIF4 in thermomorphogenesis. Interestingly, the *hda19* mutation induced a clear increase of *PIF4*, as well as *IAA29* and *YUC8* expression at 27 °C **(Figure 5)**, suggesting that HDA9 and HDA19 may have different functions in temperature-inducible gene expression. This was surprising given that *hda19* mutants failed to induce high temperature-mediated hypocotyl elongation, although high temperature-induced decrease of *HY5* expression was strongly attenuated in *hda19* compared with wild type seedlings. These data indicated that that HDA19 may regulate thermosensory hypocotyl growth through a distinct signaling pathway and HDA15 was not involved in gene expression regulated by the HY5 PIF4 pathway, whereas HDA9 positively regulated the induction of PIF4-activated downstream gene expression upon elevated temperature.

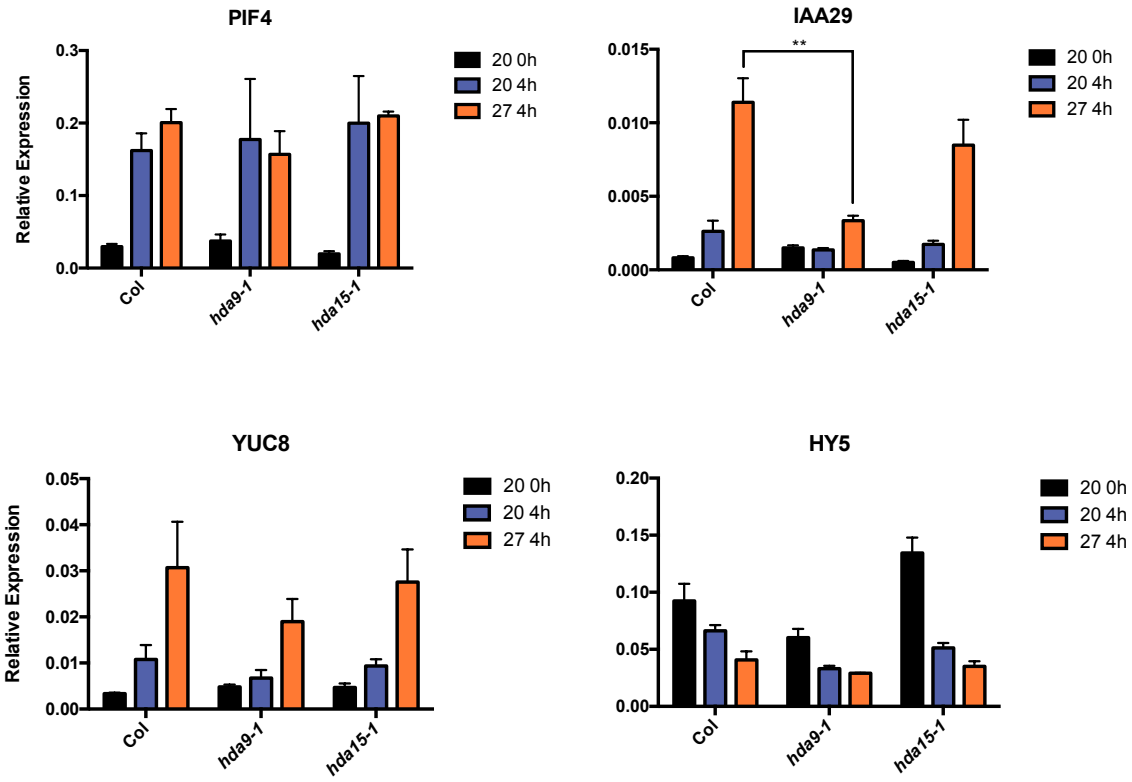

Figure 4. Expression analyses of PIF4-HY5-mediated signaling pathway in response to elevated temperature in hda9-1 and hda15-1 mutants

Expression of growth-related genes *PIF4*, *IAA29*, *YUC8* and *HY5* in Col-0, *hda9-1* and *hda15-1* mutants as measured by qRT-PCR (mean ± SD of three biological replicates) in 10-day-old seedlings grown constantly at 20 °C, or incubation at 27 °C for 4 hr in SD conditions. **P <0.01 (Student's t test) significantly different from Col-0 in corresponding temperature conditions. Primers were shown at **Supplemental table 2.**

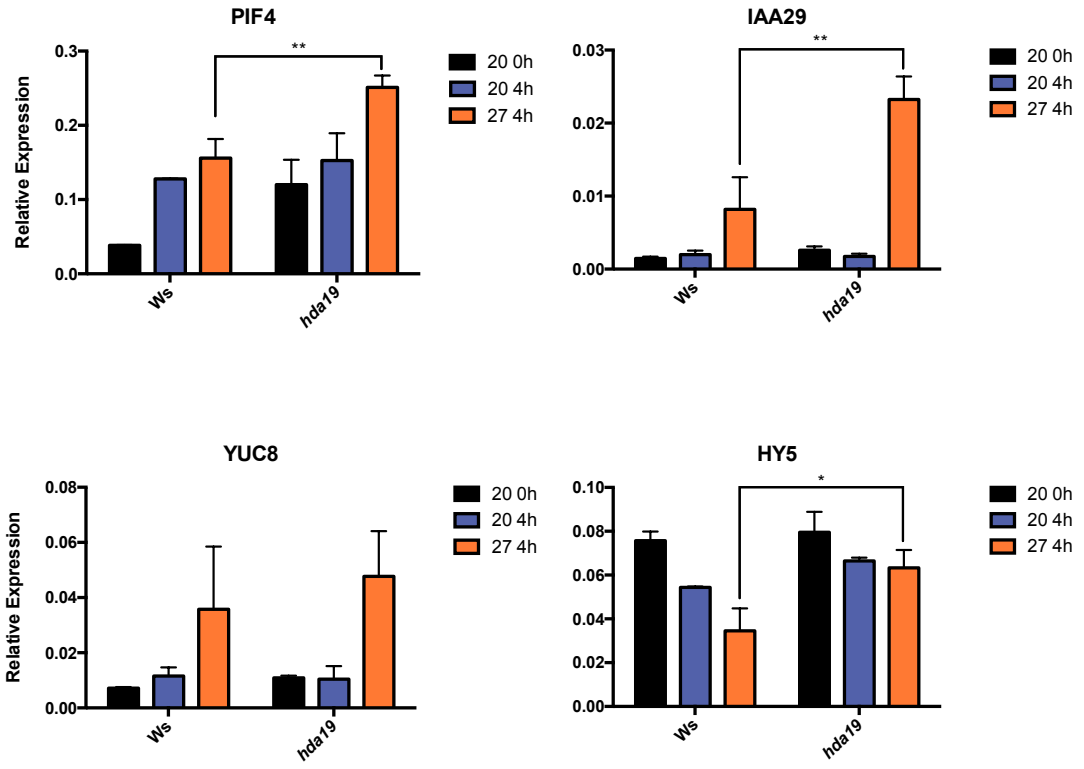

Figure 5. Expression analyses of PIF4-HY5-mediated signaling pathway in response to elevated temperature in hda19 mutants

Expression of growth-related genes *PIF4*, *IAA29*, *YUC8* and *HY5* in Ws, *hda19* mutants as measured by qRT-PCR (mean ± SD of three biological replicates) in 10-day-old seedlings grown constantly at 20 °C, or incubation at 27 °C for 4 hr in SD conditions. *P <0.05, **P <0.01 (Student's t test) significantly different from Ws in corresponding temperature conditions. Primers were shown at **Supplemental table 2.**

Genome-wide identification of warm temperature-induced gene expression changes in HDACs mutants

To further evaluate the effects of HDACs on gene expression in warm temperature condition, we analyzed the transcriptomes of 10-day-old seedlings of wild type (Col-0 and Ws) and the HDACs mutants *hda9-1, hda15-1* and *hda19* after shifting from 20 to 27 °C for 4 hr by RNA-seq. Two biological repeats with mRNA isolated from

different seedlings were performed. Trimmomatic (version 0.32), TopHat (version 2.0.13), and Cufflink pipelined software were used to identify differentially expressed genes; above 55% raw tags were of high quality, of which above 76% could be aligned to the reference *Arabidopsis* genome **(Figure S3A)**. The sequences of the biological repeats of each genotypes displayed a high reproducibility (R^2>0.962, **Figure S3B**). These parameters confirmed the high quality of the RNA-sequencing data. The RNA-seq revealed that the *hda15-1, hda9-1,* and *hda19* mutations affected large numbers of genes at 20 °C and 27 °C compared to their respective wild type **(Figure 6A)**. Higher numbers of dysregulated genes were found in *hda9-1* at 20 °C and in *hda19* at 20 °C and 27 °C. The dysregulated genes in the mutants at 20 °C did not overlap **(Figure 6B)**, confirming distinct function of these HDACs in gene regulation.

We then analyzed the differential expression patterns of wild type and mutant lines at different temperatures **(Figure 6C**). Genes with more than 2 times of difference between comparing conditions were assigned as differentially expressed (DEGs). The expression levels of 639 and 1144 genes (251 and 626 up-regulated and, 379 and 518 down-regulated) changed in Col-0 and Ws, respectively, after the seedlings were shifted from 20 to 27 °C for 4 hr. In *hda9-1* and *hda19* mutants, 645 and 1116 DEGs were detected, 456 and 606 were up-regulated and 189 and 510 were down-regulated in *hda9-1* and *hda19* mutants, respectively. In *hda15-1* mutants, the shifting from 20 to 27 °C for 4 hr dramatically affected genes expression, since 529 down- and 733 up-regulated DEGs were identified in *hda15-1* seedlings, suggesting that HDA15 played an important role in gene expression in responding to elevated temperature. The gene expression changes between wild types and the mutants induced by the 20 °C to 27 °C shift was largely different **(Figure 6D)**, suggesting that the mutations affected different gene expression programs of warm-temperature responses.

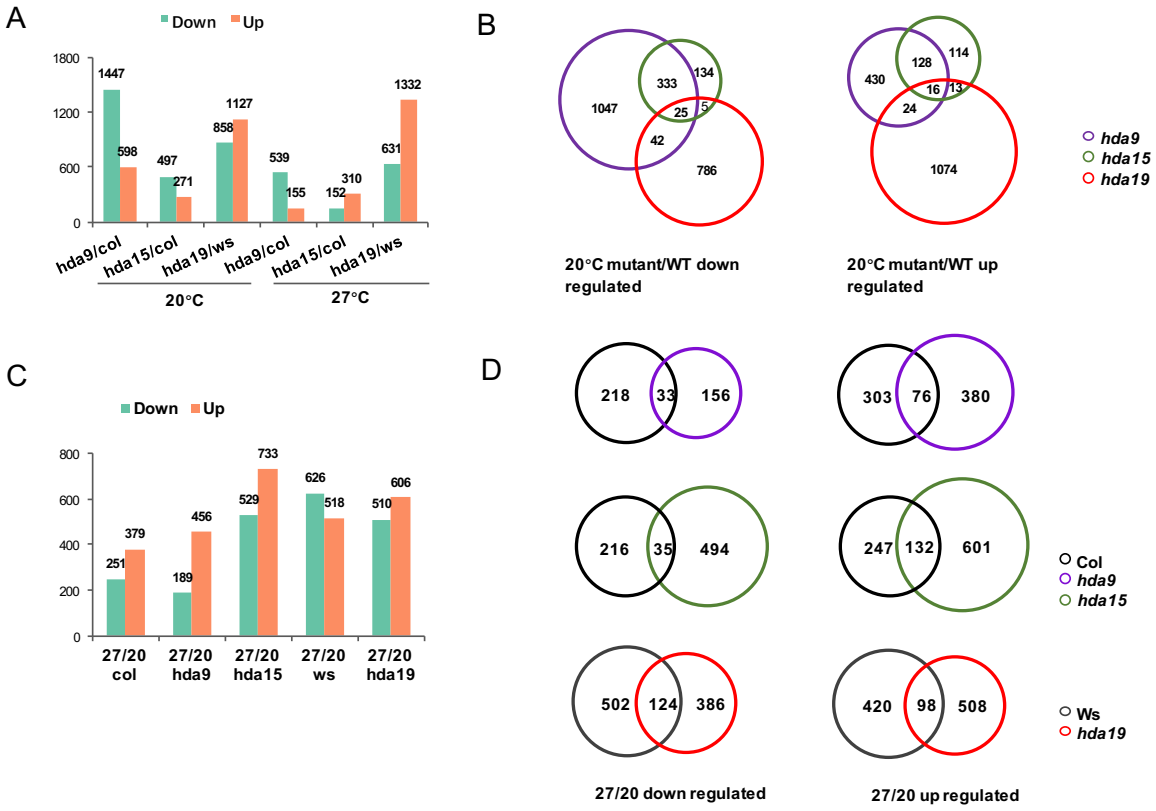

Figure 6. Analysis of temperature-specific genome-wide transcript profile of HDACs mutants

(A) Numbers of DEGs in HDACs mutants compared with wild type seedlings at specific temperature conditions.

(B) Overlapped DEGs between HDACs mutants at 20 °C.

(C) Numbers of DEGs in HDACs mutants and wild type seedlings after shifted from 20 to 27 °C for 4 hr.

(D) Overlapped DEGs between HDACs mutants and corresponding wild type seedlings after shifted from 20 to 27 °C for 4 hr.

Loss-of-function of HDA9, HDA15 and HDA19 preferentially induces the expression of genes related to primary and cellular metabolic process and/or stress-responses

Gene ontology analysis revealed that up-regulated genes in *hda9-1* at 20 °C were enriched for metabolism and stress response **(Figure 7A)**. However, the enrichment became less clear at 27 °C **(Figure 7B)**. Up-regulated genes in *hda15-1* at 20 °C and 27 °C were mostly enriched in plant primary metabolism, while that in *hda19* at both temperatures were mainly enriched for stress responses **(Figure 7)**. The analysis suggested that HDA19 is involved in the regulation mainly of stress-responsive genes under normal and higher ambient temperatures, while HDA15 may influence plant thermomorphogenesis by particularly regulating metabolic process under warm temperature.

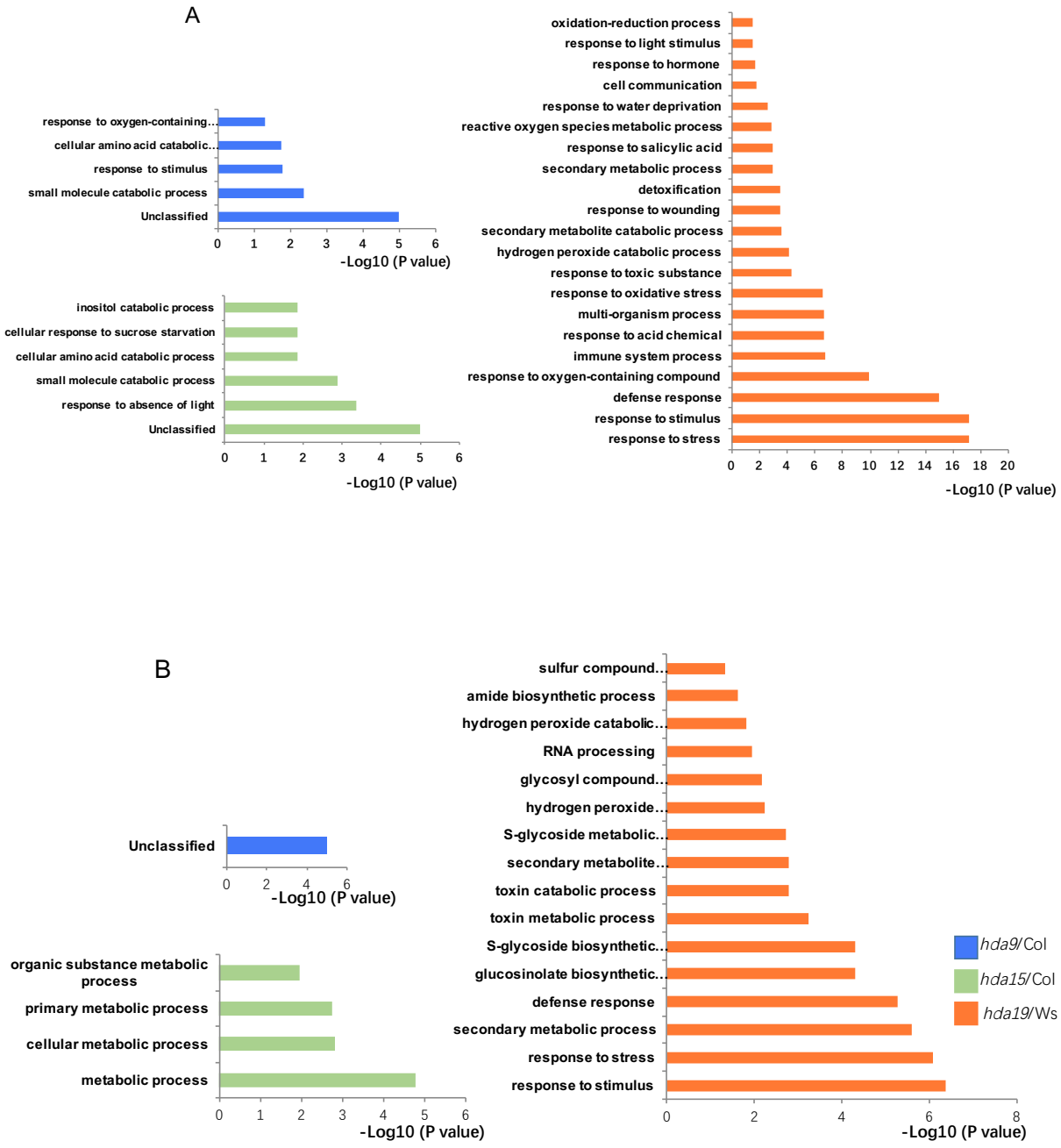

Figure 7. GO enrichment of up-regulated DEGs in HDACs mutants

GO enrichment analysis of up-regulated DEGs in *hda9* (Blue), *hda15* (Green) and *hda19* (Orange) mutants compared with their corresponding wild type seedlings at 20 °C (A) or after shifted from 20 to 27 °C for 4 hr (B).

HDA9 and HDA19 act differently in the regulation of warm temperature response

We next studied the GO enrichment of *hda9-1* mutants under warm temperature conditions. Although up-regulated DEGs in *hda9-1* mutants cannot be classified **(Figure 7B)**, several down-regulated DEGs can be clearly grouped. For examples, 10 genes of SAUR-like auxin-responsive protein family and 3 indole-3-acetic acid inducible genes were found in these genes **(Table S1)**, suggesting that HDA9 might be required for the expression of auxin response-related genes in warm temperature. In addition, 9 leucine-rich repeat protein kinase family protein, 9 cytochrome, 6 glutathione S-transferase superfamily, 4 protein kinase superfamily protein and 3 cysteine-rich RLK (RECEPTOR-like) protein kinase genes were also found to be among the down-regulated DEGs in *hda9-1* mutants, suggesting other function of HDA9 in warm temperature conditions. Furthermore, we observed that more than 50% of up-regulated DEGs at 20 °C and 27 °C were overlapped in *hda19* mutants **(Figure S4D**). So we next asked which groups of up-regulated DEGs were responsible for warm temperature responses in *hda19* mutants. GO enrichment analysis was performed in these genes. As shown in **Figure S4A**, 583 20 °C-specific up-regulated DEGs were found to enrich in the stress responses process. Also, 544 overlapped up-regulated DEGs belonged to the stress responses process (**Figure S4B)**. However, 788 specific up-regulated DEGs under warm temperature were not enriched in the stress responses process, but in the secondary metabolism process and DNA replication (**Figure S4C)**, suggesting that HDA19 might indirectly regulate plant adaptation for warm temperature through controlling the stress responses. Together, these data indicate that HDA9 and HDA19 play distinct roles in responding to ambient temperature.

3.2.4 Discussion

Histone acetylation is a highly dynamic and reversible process which is suggested to play an important role in gene expression to allow rapid adaptation of plants to environmental variations, as light, temperature, and stresses (Servet et al., 2010). Histone acetylation enzymes have been shown to be essential for plant responses to light and to stresses (Gu et al., 2017; Ha et al., 2017; Ueda et al., 2017; Zhang et al., 2017). However, their function in plant responses to higher ambient temperature has not been demonstrated. In this work, we show that although belonging to the same HDAC subfamily, histone deacetylases HDA9, HDA15, and HDA19 have distinct function in plant responses and gene expression to higher temperature. Our data indicate that HDA15 is likely to repress plant response to elevated temperature, while HDA9 and HDA19 stimulate the process. The different responses to higher temperature suggest that the 3 HDACs regulate different signaling pathways and target to different genes. The later hypothesis is supported by the transcriptomic data. The observed differences may also be related to their differences in expression, cellular compartmentalization, and interaction with other proteins/regulators.

It was shown that HDA15 interacts with PIF3 to repress chlorophyll biosynthesis genes in the dark (Liu et al., 2013). The negative function of HDA15 in higher temperature responses suggests that this histone deacetylase may repress gene expression activated by the PIF4 pathway. The repression of higher temperature-induced hypocotyl elongation by HDA15 is consistent with recent results showing that HDA15 in the light targets the promoters of a set of hypocotyl elongation-related genes, and modulate the levels of histone H4 acetylation on the associated chromatins, thus repressing gene expression (Tang et al., 2017). However, our qRT-PCR and transcriptomic data seemed not support this hypothesis. Rather, our analysis indicated that *hda15-1* mutation mainly affected primary metabolism at both normal and higher ambient temperatures. Our data suggests that metabolic activity repressed by HDA15 is essential for plant response to higher temperature. How HDA15 responds to warm temperature signal and regulates metabolic gene expression await further analysis.

By contrary, our data suggest that HDA19 and HDA9 appeared to play a positive role in plant responses to warm temperature. It has been shown that HDA19 functions as a general gene transcriptional repressor (especially by interacting with the general transcriptional repressor TPL/TPR) being involved in many aspects of plant development and stress-responses (Krogan et al., 2012; Long et al., 2006; Ryu et al., 2013). The enrichment of stress-responsive genes from up-regulated ones in *hda19* at both 20 °C and 27 °C corroborate its important functions in general stress-response, and suggests that HDA19 may regulate plant responses to higher temperature mainly through repression of this categories of genes. As described in the first part of this thesis, we have shown that *hda19* mutation increased cellular oxidative metabolites, which perhaps is related to responses to warm temperature. Data from qRT-PCR showing that *PIF4* and its downstream genes were de-repressed. However, this expression pattern does not help to explain the reduced hypocotyl elongation of the mutant in responding to warm temperature. Perhaps the induced expression of PIF4 and its targets was to compensate to reduced responses to warm temperature? The function of HDA19 in warm temperature-induced hypocotyl elongation seems to be opposite to its role in photomorphogenesis in which HDA19 antagonizes GCN5 in promoting light-regulated gene expression and hypocotyl repression (Benhamed et al., 2006). The observations suggest that light-dependent and warm-temperature-induced hypocotyl elongation may involve different regulatory pathways, at least at the level of downstream genes expression, which is supported by our observation that light and temperature-dependent hypocotyl elongation shows synergistic effect at SD condition.

Although *hda9* displayed a similar phenotypical behavior as *hda19* in warm temperature response, this HDAC likely regulates a different set of genes. The qRT-PCR data rather suggests a requirement of HDA9 for PIF4 downstream gene (e.g. IAA29, perhaps YUC8) expression. Considering that HDA9 is a presumably a transcriptional repressor, the down-regulation of PIF targets in *hda9* mutant suggests an indirect effect of *hda9* mutation. Although we observed that *hda9* mutation also resulted in up-regulation of stress-related and metabolic genes at 20 °C, at 27 °C, there were relatively few de-regulated genes compared to *hda19*. Perhaps, gene expression changes in the mutant at normal temperature already contributed to reduced sensitivity to warm temperature. HDA9 has been shown to deacetylate H3K27 to regulate flowering time, aging, and stress tolerance (Kim et al., 2013;

Zheng et al., 2016; Chen et al., 2016). The present data add an additional role of this HDAC, indicating that HDA9 plays also an important role in plant growth. Interestingly, Zheng et al (2016) have shown that, unlike HDA19 and HDA6 that plays a positive role in stress-responses, HDA9 actually represses stress-responses and tolerance. Therefore, HDACs may agonistic or antagonistic function in regulation of plant growth.

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3.2.6 Supplemental data

3.2.6.1 Supplemental figures

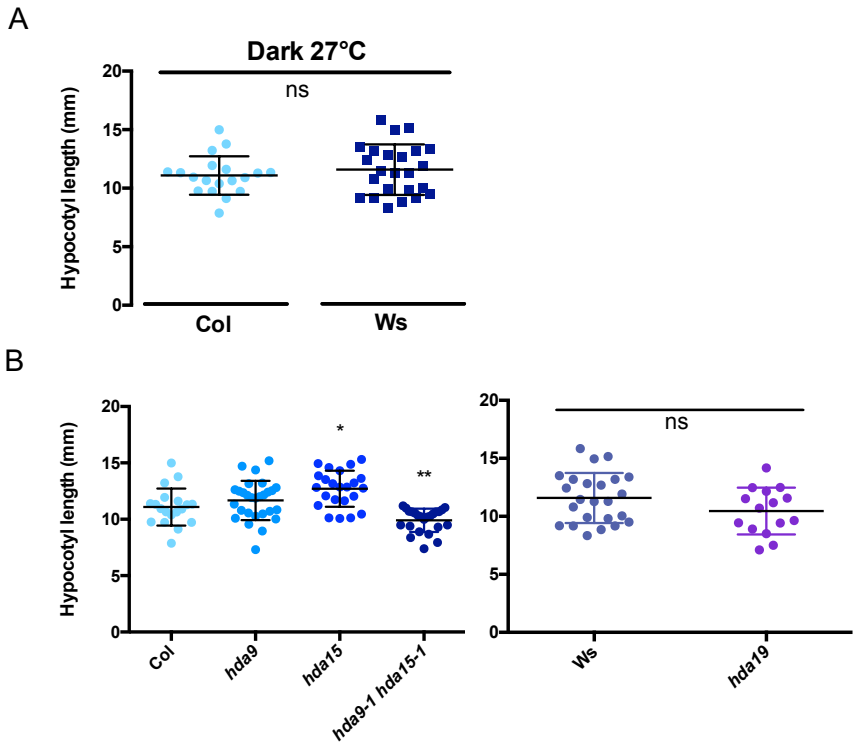

Figure S1. Thermosensory hypocotyl growth in dark condition, related to Figure 1

(A) 10-day-old seedling hypocotyl lengths of Col-0 and Ws grown in darkness at 27 °C.

(B) Hypocotyl length of 10-day old dark-grown mutants seedling. Left, comparison of the *hda9-1* and *hda15-1* alleles, as well as the double mutant *hda9-1hda15-1* with the wild type (Col-0); right, comparison of *hda19* mutant and corresponding wild type (Ws). Means of at least 15 plantlets are given. Error bars represent SD values. The measures were analyzed by Student's t test at *P<0.05 and **P <0.01. NS, no significant differences

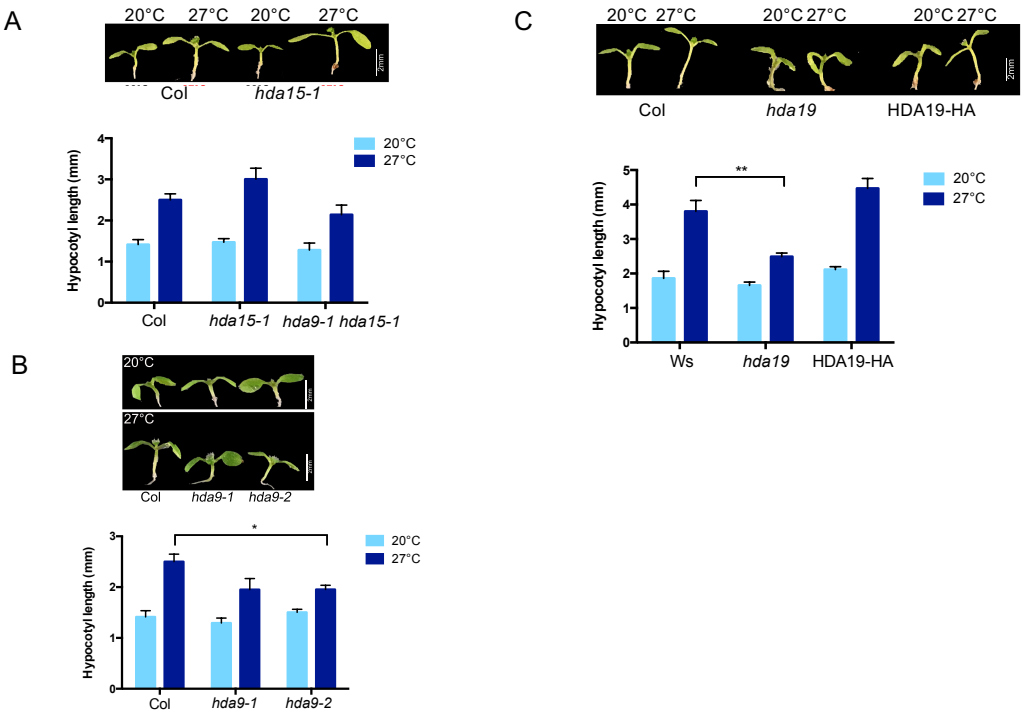

Figure S2. Control of thermosensory hypocotyl growth by HDA9 and HDA15, related to Figure 2

10-day-old seedling phenotypes and hypocotyl measurement data (mean ± SD; n≥20) of *hda15-1* alleles and *hda9-1hda15-1* double mutants (A), *hda9-1* and *hda9-2* alleles (B), and *hda19* mutants and HDA19-HA complementary transgenic seedlings (C) grown at constant 20 and 27 ºC under LD condition. *P≤0.05, **P≤0.01 (Student's t-test) significantly different from corresponding wild type in corresponding temperature conditions.

A

	Repeat	Raw read	Trimmed reads	Concordant alignment reads
20-col	1	24707109	17747608 (71.83%)	15149852 (85.36%)
	2	35693831	25649840 (71.86%)	21927350 (85.49%)
20-hda9	1	45108806	30269494 (67.10%)	25717337 (84.96%)
	2	34365194	22978061 (66.86%)	19500265 (84.86%)
20-hda15	1	28096253	20944009 (74.54%)	17806839 (85.02%)
	2	32602090	22462524 (68.90%)	19258114 (85.73%)
20-ws	1	49351342	34120093 (69.14%)	26130317 (76.58%)
	2	36758346	26345300 (71.67%)	20635993 (78.33%)
20-hda19	1	31822587	20022850 (62.92%)	16085476 (80.34%)
	2	33018155	23562239 (71.36%)	19072306 (80.94%)
27-col	1	30322040	22503911 (74.22%)	19154530 (85.12%)
	2	34542624	24501291 (70.93%)	20683466 (84.42%)
27-hda9	1	27472296	20015818 (72.86%)	16772146 (83.79%)
	2	30713551	21507048 (70.02%)	17980105 (83.60%)
27-hda15	1	32896055	23684256 (72.00%)	19867941 (83.89%)
	2	27212885	20738272 (76.21%)	17490399 (84.34%)
27-ws	1	35176110	26908612 (76.50%)	20619562 (76.63%)
	2	32554804	22755942 (69.90%)	16981004 (74.62%)
27-hda19	1	30151830	22590675 (74.92%)	18570307 (82.20%)
	2	37207524	20772645 (55.83%)	16439678 (79.14%)

B

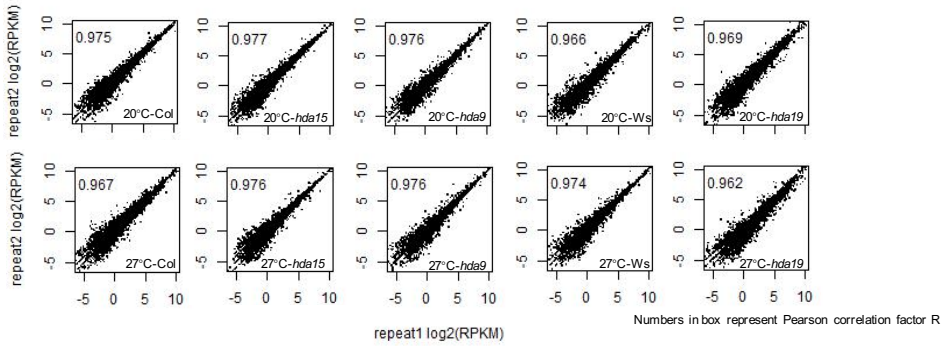

Figure S3. RNA-seq reads and reproducibility of HDACs mutants and wild type

(A) Table shows the RNA-seq reads and analysis data of HDACs mutants and wild type (Col-0 and Ws).

(B) Two-wise scatter plots of RNA-seq reads between two biological repeats. Each point represents one gene locus. RPKM, Reads Per Kilobase per Million reads. Correlation coefficient R2 indicated.

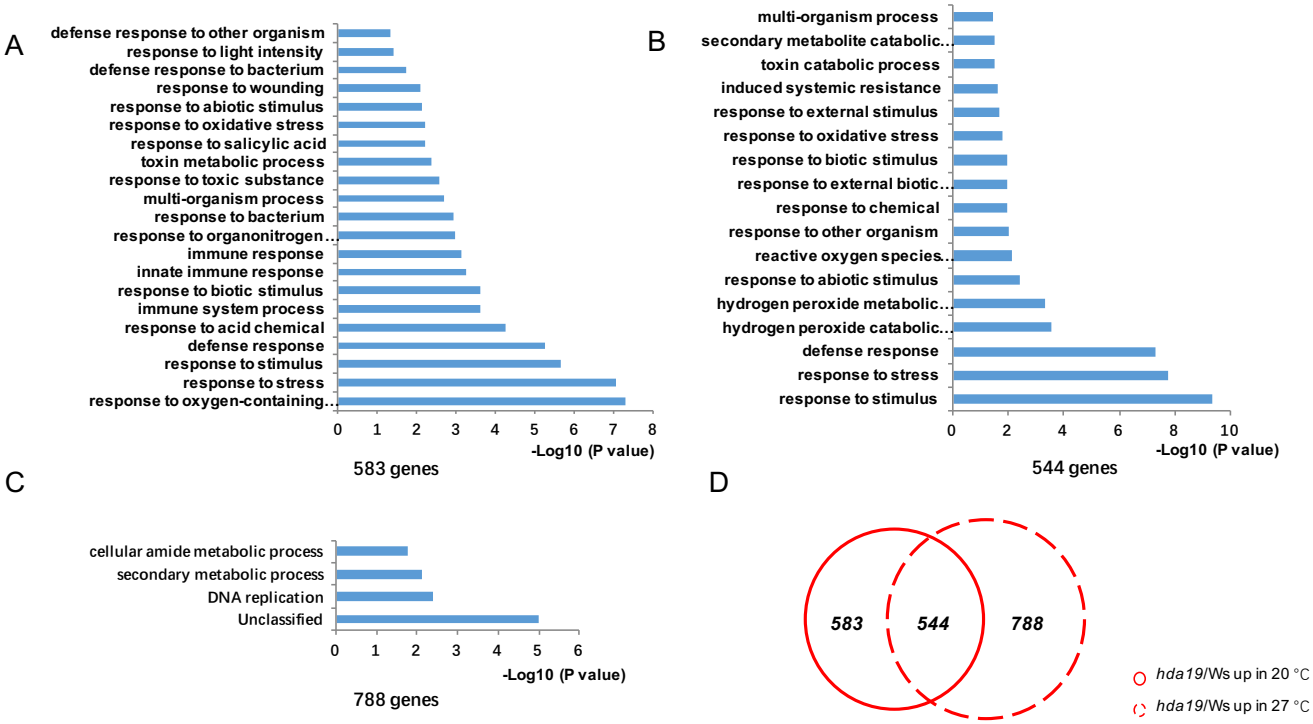

Figure S4. Functional categorization of different groups of up-regulated DEGs in hda19 mutants at corresponding temperature conditions by Gene Ontology analysis

GO enrichment of 583 20 °C-specific (A), 544 overlapped (B) and 788 27 °C-specific (C) up-regulated DEGs. (D) Overlapped up-regulated DEGs in *hda19* mutants at different temperature conditions.

3.2.6.2 Supplemental tables

Table S1. GO enrichment of down-regulated DEGs in *hda9-1* mutants at 27 ºC

Gene family	Gene ID	log2 Ratio(*hda9-1*/Col-0)	Gene annotation
Proteinkinase family	AT5G35960	-3.59024	Proteinkinase family protein
	AT4G10390	-1.59618	Proteinkinase superfamily protein
	AT1G78530	-1.16601	Proteinkinase superfamily protein
	AT1G74490	-1.1167	Proteinkinase superfamily protein
Leucine-rich repeat protein kinase family	AT1G51830	-2.23386	Leucine-rich repeat protein kinase family protein
	AT1G51820	-1.74998	Leucine-rich repeat protein kinase family protein
	AT1G05700	-1.66247	Leucine-rich repeat transmembrane protein kinase protein
	AT2G14510	-1.45775	Leucine-rich repeat protein kinase family protein
	AT2G28970	-1.44603	Leucine-rich repeat protein kinase family protein
	AT1G07560	-1.44394	Leucine-rich repeat protein kinase family protein
	AT5G49780	-1.31893	Leucine-rich repeat protein kinase family protein
	AT3G46330	-1.25716	Leucine-rich repeat protein kinase family protein
	AT2G28960	-1.22655	Leucine-rich repeat protein kinase family protein
Cysteine-rich RLK (RECEPTOR-like protein kinase)	AT4G23220	-1.54324	cysteine-rich RLK 14
	AT4G05200	-1.30023	cysteine-rich RLK 25
	AT4G11530	-1.07923	cysteine-rich RLK 34
Glutathione S-transferase	AT1G49860	-3.54106	glutathione S-transferase (class phi) 14
	AT5G02780	-2.3554	glutathione transferase lambda 1
	AT5G17220	-1.59143	glutathione S-transferase phi 12
	AT1G78370	-1.40313	glutathione S-transferase TAU 20
	AT3G09270	-1.22849	glutathione S-transferase TAU 8
	AT3G03190	-1.0681	glutathione S-transferase

			F11
Cytochrome P450	AT3G48290	-2.38487	cytochrome P450, family 71, subfamily A, polypeptide 24
	AT2G30770	-1.54362	cytochrome P450, family 71, subfamily A, polypeptide 13
	AT1G01600	-1.429	cytochrome P450, family 71, subfamily A, polypeptide 13
	AT5G23190	-1.42609	cytochrome P450, family 86, subfamily A, polypeptide 4
	AT3G26160	-1.27662	cytochrome P450, family 86, subfamily B, polypeptide 1
	AT4G15393	-1.08216	cytochrome P450, family 702, subfamily A, polypeptide 5
	AT4G31940	-1.08039	cytochrome P450, family 82, subfamily C, polypeptide 4
Indole-3-acetic acid (IAA)	AT1G15050	-1.95287	indole-3-acetic acid inducible 34
	AT1G52830	-1.84632	indole-3-acetic acid 6
	AT4G32280	-1.14976	indole-3-acetic acid inducible 29
SAUR-like auxin-responsive protein family,	AT5G50760	-1.58129	SAUR-like auxin-responsive protein family, SAUR67
	AT5G18010	-1.57236	SAUR-like auxin-responsive protein family, SAUR19
	AT5G18060	-1.46296	SAUR-like auxin-responsive protein family, SAUR23
	AT1G17345	-1.28198	SAUR-like auxin-responsive protein family, SAUR77
	AT3G03840	-1.24881	SAUR-like auxin-responsive protein family, SAUR27
	AT1G29510	-1.22822	SAUR-like auxin-responsive protein family, SAUR67
	AT1G29460	-1.12771	SAUR-like auxin-responsive protein family
	AT1G29450	-1.10695	SAUR-like auxin-responsive protein family
	AT3G53250	-1.10081	SAUR-like auxin-responsive protein family
	AT5G18020	-1.00502	SAUR-like auxin-responsive protein family, SAUR20

Table S2. Sequences of the primers used in this study

RT-qPCR	Forward primer sequence	Reverse primer sequence
YUC8	CGATGAGACCAGTGGCTTGT	TTTTCTCCCGTAGCCACCAC
HY5	CATCAAGCAGCGAGAGGTCA	CCGACAGCTTCTCCTCCAAA
PIF4	ACCTCAGAGACGGTTAAGCC	TGGAGGAGGCATGACTTGAG
IAA29	ATCACCATCATTGCCCGTAT	TTGCCACACCATCCATCTTA

CONCLUSION AND PERSPECTIVE

4.1 SA-mediated redox modification regulates HDA19 function in *Arabidopsis*

HATs/HDACs are important regulators and play essential roles in many plant development processes and responses to environmental changes. Although it was reported that some HATs/HDACs have co-factors to regulate acetylation/deacetylation function or these enzymes can be posttranslationally modified, the regulatory mechanism of HATs/HDACs functions remain largely unknown, especially in plants. Previous results observed in the lab showed that a phosphatase 2C protein as an interacting partner of the GCN5 negatively regulate its HAT activity in *Arabidopsis* (Servet et al., 2008). Using a modified S-nitrosylation switch assay, we here first demonstrate that HDA19 is S-nitrosylated *in vivo* and *in vitro* upon SA and GSNO treatment, respectively. In addition, confocal imaging analysis show that HDA19 exists in both nucleus and cytoplasm, and HDA19 nuclear accumulation is significantly decreased upon SA treatment. Consistent with this redox modification and decreased nuclear accumulation of HDA19, SA treatment leads to an enhancement of global histone acetylation. We further show that loss-function of HDA19 results in induction of several redox-related genes expression. The contents of $NAD/NADH^{+}$ and GSH/GSSH are also increased in the *hda19* mutants. Thus, these data together suggest that HDA19 and SA-signaling seem to form a negative loop to regulate cellular redox states and gene expression to sense environmental conditions to control plant growth. Accordingly, I propose this model **(Fig 14)**:

In nonstressed conditions: HDA19 help to establish a repressive chromatin state by deacetylation of histones at stress genes.

Under stress conditions: stress perception initiates SA production, which enhances ROS accumulation and form a self-amplifying feedback loop between ROS and SA. Cellular redox changes negatively regulate the HDA19 nuclear accumulation through

a reversible S-nitrosylation, thereby enhancing histone acetylation and promoting a supportive chromatin state for the expression of stress genes. At the same time, increased GSH and NAD^+ contents caused by HDA19 degradation might contribute to the cell redox homeostasis.

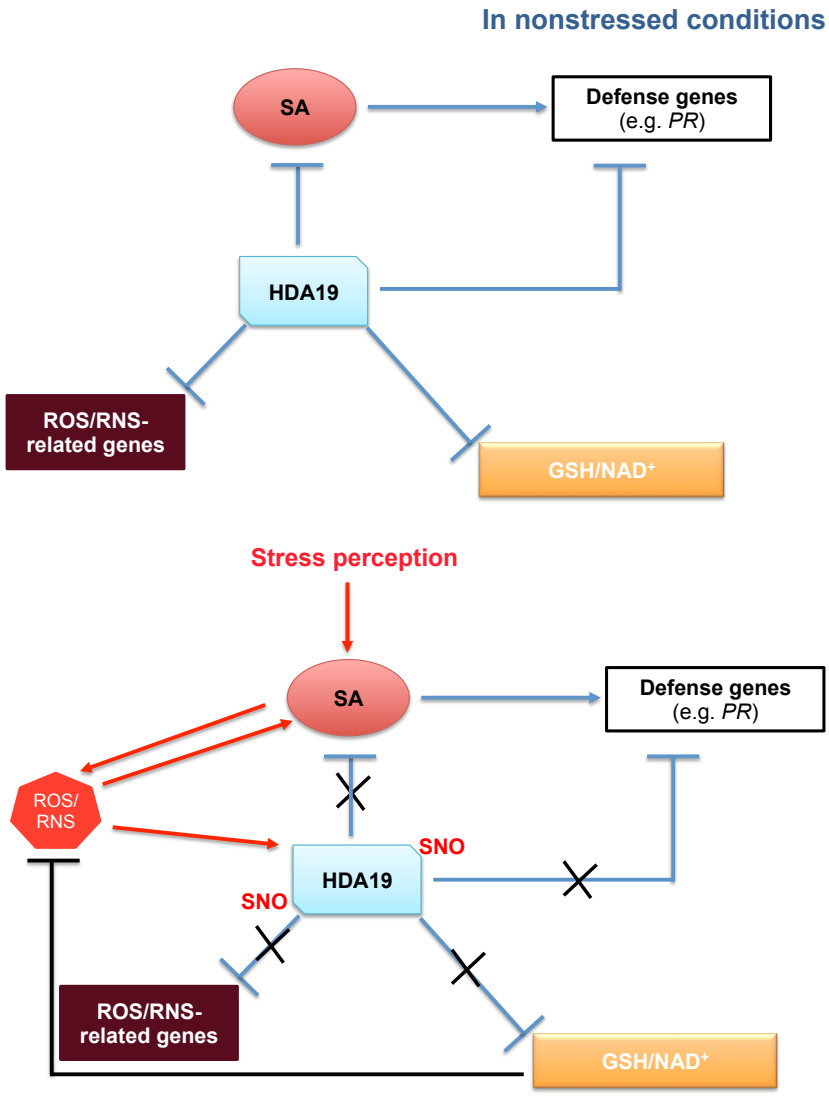

Figure 14. Proposed model of HDA19 functions in SA-mediated stress responses signaling pathway

Owing to the rapid progress of liquid chromatography-tandem mass spectrometry and sequencing analysis techniques, it will be very important to detect the SA-mediated specific S-nitrosylated Cys residues of HDA19 and their effects on regulation of HDA19 location, stability as well as HDACs activities. Moreover, the HDAC activity of S-nitrosylated HDA19 could be evaluated *in vivo* or *in vitro* to study the role of redox modification in promoting stress-induced transcription of genes through epigenetic machinery. Finally, it will be of great importance to study the interplay between S-nitrosylated HDA19 and other co-factors (such as related-transcription factors, HDAC complexes, etc.) in SA-mediated signaling pathways related to plant development and stress responses.

4.2 The functions of HDACs in warm temperature responses in *Arabidopsis*

Projected global warming poses a serious threat to agricultural productivity since even small increases in ambient temperature considerably hampers plant productivity. However, although a large body of evidences is available on how plants cope with heat stress, little is known about the regulatory mechanism that plants respond to moderate increased temperatures. Using the model plant *Arabidopsis*, we here identify the HDAC proteins, HDA9, HDA15, and HDA19, as the key temperature regulator of elongation growth resulting in thermomorphogenesis. HDA15 negatively regulates the hypocotyl elongation under warm ambient temperature condition, whereas HDA9 and HDA19 promote the hypocotyl elongation in response to the elevated temperature. Moveover, gene expression and RNA-seq analysis of young seedlings shifted from 20 to 27 °C for 4 hr revealed that HDA9, HDA15, and HDA19 display distinct functions in the regulation of gene expression; HDA15 affects primary metabolic and cellular metabolic process under warm temperature conditions;

HDA9 may operate downstream of auxin action and specific protein kinases signaling pathways; HDA19 might be a coordinator that integrates thermosensory growth and stress-response or immunity in *Arabidopsis* **(Fig 15)**. However, the precise mechanisms involving these HDAC in warm-temperature sensing and gene regulation remain to be studies in the future.

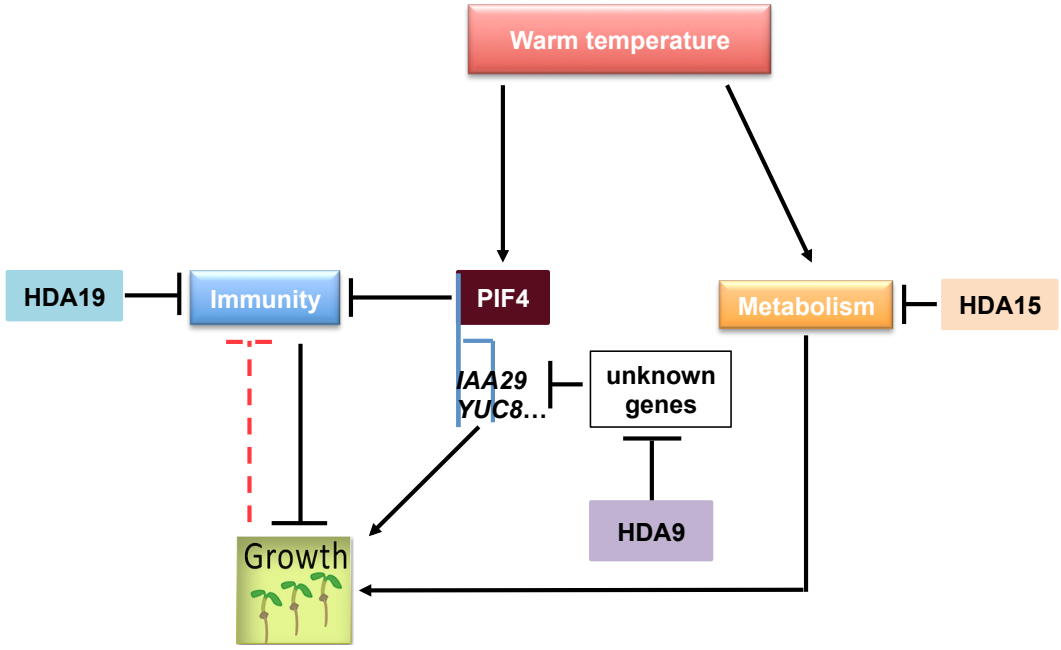

Figure 15. Schematic representation of the functions of HDACs in warm temperature responses in *Arabidopsis*

HDACs remove acetyl epigenetic marks from nucleosomal histones, resulting in local chromatin compaction, thereby limiting access for transcription factors and the transcription machinery. HDACs enzymatic activity is non-specific and HDACs enzymes are incapable of direct DNA binding. Therefore, HDACs enzymes must be recruited to their target genes by specific DNA-binding factors, including transcription factors and/or repressors that are part of the HDACs-multiprotein complex. However, the multiprotein complexes in which HDA9, HDA15 and HDA19

operate under warm temperature conditions are unknown, except that HDA19 functions in the transcriptional repressive complex involving TPL. Thus, the next step of this study will be elucidation of these HDACs-multiprotein complexes using advanced proteomics to detect temperature-specific factors and study their role in thermomorphogensis in molecular detail. Moreover, HDACs control local gene expression by specifically associating to the chromatin of their target genes. Therefore, genes that contribute to HDA9-, HDA15- and HDA19-dependent thermomorphogenesis are expected to be targeted by these HDACs-related multiprotein complexes and differentially acetylated and expressed in a HDACs-dependent manner under high ambient temperature conditions. Identification of these HDACs-multiprotein complexes target genes will be great of important to further characterize the thermosensory responses in plants. ChIP-seq analysis will be carried out to test the selected candidate genes from our RNA-seq date. Overlaying the datasets by bioinformatics means will reveal novel genes that are directly targeted by HDA9, HDA15 and HDA19, as well as their multiprotein complexes partners and it will be useful to establish the role of these targets in thermomorphogenesis.

RÉSUMÉ DE LA THÈSE EN FRANÇAIS

Au cours de ces dernières années, la découverte des mécanismes de régulation épigénétiques a suscité un intérêt sans précédant dans le domaine de la recherche académique, mais également comme ouverture au développements d'applications bénéfiques dans le domaine de la biologie végétale. Les recherches concernant les régulateurs épigénétiques des plantes ont montré qu'un certain nombre de modifications épigénétiques régulaient de nombreux processus développementaux, mais également la réponse au stress. Les recherches destinées à décrypter les mécanismes possibles de régulation des processus physiologiques essentiels pour les plantes, ainsi que la caractérisation des changements phénotypiques résultant de modifications épigénétiques, devrait permettre d'améliorer l'adaptation à long-terme des plantes aux changements environnementaux, ouvrant des nouvelles stratégies pour augmenter la productivité.

L'acide salicylique est l'un des régulateurs les plus polyvalents et, en tant que phytohormone, joue un rôle crucial dans de nombreuses voies de développement et dans les réponses au stress. Les fonctions de l'acide salicylique dans l'immunité des plantes ont été largement caractérisées et disséquées en utilisant des approches génétiques et biochimiques. Par exemple, les plantes mutantes qui sont altérées dans la synthèse de l'acide salicylique ont échoué à exprimer les gènes liés à la pathogenèse lors d'une infection pathogène et les résistances pathogènes des lignées mutantes ont été gravement compromises. En revanche, il a été démontré que l'acide salicylique

provenant de sources exogènes pour les plantes améliore la résistance des plantes à l'infection par les agents pathogènes.

Des preuves récentes ont montré que les voies de signalisation médiées par l'acide salicylique interagissaient avec la signalisation redox pour répondre aux stress environnementaux. Il a été montré que NONEXPRESSER OF PR GENES 1 (NPR1), acteur clé fonctionnant en aval de l'acide salicylique dans la réponse de défense de la plante, modifiait son état redox en réponse à l'accumulation d'acide salicylique endogène ou au traitement exogène par l'acide salicylique. De plus, les modifications de l'état redox de la cellule modulent la synthèse de l'acide salicylique, régulant l'expression des gènes de résistance aux pathogènes. Cependant, bien que le lien entre les voies de signalisation médiées par l'acide salicylique et la signalisation rédox impliquée dans la réponse au stress aient été identifiées et, dans une certaine mesure caractérisées, les détails du mécanisme de la communication croisée entre ces deux facteurs restent largement inconnus.

L'homéostasie de l'acétylation des histones est contrôlée par les histones acétyltransférases (HAT) et les histones déacétylases (HDAC) qui catalysent le transfert et l'élimination des groupes acétyles de l'acétyl-CoA aux résidus de lysine (K) N-terminale des protéines des queues d'histone. Un HDAC RPD3 / HDA1 de classe I, HDA19 s'est avéré être un régulateur transcriptionnel global qui fournit une régulation des gènes qui est réversible en réponse aux changements dans les

programmes de développement et les processus physiologiques. Zhou et al ont montré que HDA19 et HSL1 peuvent agir ensemble pour réprimer l'expression des gènes de maturation des graines pendant la germination. D'autres recherches ont confirmé que l'invalidation ou la surexpression de HDA19 entraînaient de multiples défauts de croissance et de développement tels que le développement de feuilles anormales, une floraison retardée et une fertilité réduite. HDA19, ainsi que HDA6, contribuent ensemble à la répression des propriétés embryonnaires après la germination. De plus, il a été montré que HDA19 interagissait avec SCL15 pour contrôler le programme de maturation des graines. HDA19 a également été impliqué dans les systèmes de défense. Il a d'abord été rapporté que HDA19 participait à la régulation des réponses de défense médiées par les voies de signalisation éthylène / acide jasmonique, car la surexpression de HDA19 régule à la hausse plusieurs gènes induits par l'éthylène / acide jasmonique et par la résistance à un pathogène fongique. En outre, il a été proposé que HDA19 soit impliqué dans les réponses de défense à médiation par l'acide salicylique via une régulation positive de l'expression du gène 1 liée à la pathogenèse. Bien que HDA19 joue des rôles importants dans plusieurs processus de développement et de physiologie d'Arabidopsis, on ignore si HDA19 est impliqué dans l'intégration des réponses au stress et du statut redox pour réguler l'expression des gènes et comment l'activité HDA19 est elle-même régulée.

Au cours des dernières années, un certain nombre de preuves ont mis en évidence l'existence d'interaction entre l'acétylation des histones, les réponses au stress et l'homéostasie redox. Les espèces réactives de l'oxygène ou de l'azote sont maintenant considérées comme jouant leurs rôles vraisemblablement par des modifications oxydatives des protéines qui sont sensibles à la perturbation des états redox cellulaires. En conséquence, des changements dans l'équilibre redox cellulaire agissent comme un signal efficace qui informe la cellule des conditions environnementales dominantes pour réguler l'activité des histones acétylates / désacétylases. Après l'intégration de ces informations par la cellule, celle-ci est capable d'engager une réponse appropriée via la reprogrammation de l'expression du gène nucléaire. Chez les mammifères, le stress oxydatif peut modifier la conformation des HDAC, entraînant une altération de leur localisation subcellulaire ou une réduction de leur activité catalytique. Il a été montré que la réduction de Cys-667 / Cys-669 dans HDAC4 inhibait son exportation nucléaire, indépendamment de son statut de phosphorylation. En outre, le stress oxydatif diminue l'activité de HDAC1 par des modifications covalentes au niveau de deux résidus de cystéine conservés dans HDAC1. Chez Arabidopsis, des analyses protéomiques ont indiqué que HDA19 et HDA9 étaient des protéines sensibles à l'oxydoréduction. HDA19 et HDA9 se sont avérés être oxydés lors du traitement à l'acide salicylique, ce qui déclenche l'accumulation d'espèces réactives d'oxygène ou d'azote au cours des premières réponses cellulaires. L'oxydation de HDA19 et HDA9

pourrait les rendre inactifs, élevant ainsi l'acétylation des histones et l'expression des gènes associés. De plus, Mengel et al. ont récemment prouvé que la S-nitrosoglutathione, un donneur de NO physiologique, ainsi que la signalisation du NO induite par l'acide salicylique, inhibaient l'activité des HDAC in vivo. Bien que le rôle de HDA19 dans l'expression des gènes induite par l'acide salicylique soit établi, les mécanismes moléculaires de l'activation / inactivation de HDA19 par l'acide salicylique sont encore mal connus.

Au cours de mon travail de thèse, j'ai pu montrer que HDA19 est S-nitrosylé in vitro (par l'analyse d'extraits protéiques) et in vivo (dans les semis) à la suite d'un à la S-nitrosoglutathione ou à l'acide salicylique, qui régule négativement l'accumulation nucléaire HDA19, entraînant une hyperactylation des histones H3K9 et H3K14. Nous avons également constaté que la mutation HDA19 entraînait une régulation à la hausse de plusieurs gènes liés à l'oxydoréduction, ainsi qu'une augmentation des concentrations des métabolites centraux qui orchestrent l'homéostasie redox cellulaire des plantes. Nos données indiquent que l'accumulation et la fonction de HDA19 peuvent être modulées par les voies réactives de l'oxygène ou de l'azote, induisant ainsi un lien moléculaire entre la phytohormone, les modifications redox et la régulation épigénétique dans les réponses au stress chez Arabidopsis.

Les signaux environnementaux, en particulier la température, affectent la croissance et le développement des plantes et ont des effets dramatiques sur l'architecture des

plantes et la biomasse tout au long du cycle de vie. À leur tour, les plantes ont développé des mécanismes de régulation sophistiqués qui leur permettent de s'adapter rapidement à leur environnement défavorable. Bien qu'il existe de nombreuses études sur la façon dont les plantes font face au stress thermique, on sait encore peu de choses sur la façon dont les plantes réagissent à une élévation modérée des températures ambiantes. Il s'agit s'un champs de recherche important pour pour l'écologie et l'agriculture, pour pouvoir répondre aux préoccupations sérieuses résultant du réchauffement climatique quia débuté au siècle dernier.

Plusieurs espèces végétales ont la capacité de s'acclimater à une élévation de la température ambiante. Ces plantes induisent une croissance adaptative qui passe par une élongation des tiges et des feuilles qui lui permet d'améliorer sa capacité de refroidissement et d'éviter le flux de chaleur. Ce processus d'acclimatation est appelé thermomorphogénèse et il est essentiel pour la morphologie et le rendement des plantes. Bien que la régulation de la thermomorphogénèse à température ambiante chaude soit une activité fondamentale pour les plantes, le mécanisme moléculaire qui sous-tend ce processus est mal compris. Récemment, le facteur de transcription bHLH PHYTOCHROME INTERACTING FACTOR 4 (PIF4) a été présenté comme un point de signalisation central pour l'intégration dans la signalisation lors de la thermomorphogénèse. Les plantes mutantes pour PIF4 sont incapables d'augmenter la longueur de l'hypocotyle à des températures plus chaudes. De plus, PIF4 stimule la

production de la phytohormone auxine en se liant directement aux régions promotrices des gènes de biosynthèse de l'auxine, ce qui déclenche ensuite la croissance de l'élongation à des températures plus élevées. PIF4 a également été impliqué dans le processus d'élongation de l'hypocotyle induite par la température chaude et médiée par brassinostéroïdes. Des preuves récentes ont révélé que les signaux lumineux et thermiques communiquaient de manière croisée pour déterminer les réponses de croissance adaptatives d'une manière dépendante de PIF4. Par exemple, Gangappa et Kumar ont démontré que la voie photomorphogénique bien connue DET1-COP1-HY5 était nécessaire en amont pour l'induction de PIF4 à des températures élevées. De plus, la croissance thermosensible et les adaptations morphologiques de la plante se sont révélées être également directement liées à la suppression des défenses à des températures élevées, révélant un autre lien physiologique entre la thermomorphogénèse et l'immunité chez les plantes.

La régulation de la transcription génique implique des cofacteurs (co-activateur ou co-répresseur) capables de transduire des signaux provenant de facteurs de transcription spécifiques liés à un enhancer vers le complexe d'initiation de l'ARN polymérase. Des études in vitro ont montré que les cofacteurs de transcription étaint généralement associés à des activités de remodelage et de modification de la chromatine, tels que le remplacement d'histones canoniques par des variants spécialisés et le remodelage de la chromatine dépendant de l'ATP. Récemment, des

preuves de plus en plus nombreuses ont montré que des mécanismes épigénétiques étaient impliqués dans la régulation de la thermomorphogénèse des plantes et influencaient le développement des plantes à des températures ambiantes élevées. Kumar et Wigge ont monté que le variant de l'histone H2A, H2A.Z, était expulsé de la chromatine aux sites d'initiation de la transcription à des températures élevées. De ce fait, il s'avère que H2A contribue à la thermomorphogénèse en permettant la liaison des régulateurs transcriptionnels et de la machinerie transcriptionnelle aux gènes régulés par la température. En outre, Zha et al ont démontré que PICKLE, un facteur de remodelage de la chromatine dépendant de l'ATP, contrôlait la croissance thermosensible de l'hypocotyle chez Arabidopsis. La perte de fonction de PICKLE entraîne en effet une sensibilité réduite à la température chaude dans le processus d'allongement de l'hypocotyle. De plus, PICKLE affecte le niveau de triméthylation de l'histone H3 Lys 27 associée à l'INDOLE-3-ACETIC ACID INDUCIBLE 19 et INDOLE-3-ACETIC ACIDE INDUCTIBLE 29 et régule leur expression. Cependant, bien qu'il existe un faisceau de preuves indiquant l'importance du remodelage et des modifications de la chromatine lors de la thermomorphogénèse chez Arabidopsis, les mécanismes moléculaires de la voie de signalisation thermomorphogénique restent largement inconnus.

Les modifications épigénétiques des protéines structurales de la chromatine modifient également la structure locale de la chromatine. C'est le cas en particulier pour les

modifications chimiques des protéines histones par acétylation qui entraîne une régulation de l'expression des gènes. L'homéostasie de l'acétylation des histones est contrôlée par les histones acétyltransférases (HAT) et les histones déacétylases (HDAC). Les HDAC catalysent l'élimination des groupes acétyle des résidus de lysine (K) acétylés dans les protéines des queues d'histone. Chez Arabidopsis, 16 gènes HDAC ont été identifiés, dont 10 appartiennent à la superfamille RPD3 / HDA1, 4 à la famille HD2 et 2 à la famille SIR2. L'ablation génétique de la fonction des HDAC a montré que ces derniers jouaient des rôles divers et importants dans de nombreux aspects du développement et de la physiologie chez Arabidopsis. Nous avons déjà montré qu'un HDAC RPD3 / HDA1 de classe I, HDA19, était nécessaire au développement d'un phénotype photomorphogénique normal dépendant de l'ATP et à la transcription génique activée par la lumière, mais qu'il était également impliqué dans les réponses de défense. Nous avons également montré qu'un autre HDAC, HDA9, qui appartient aussi au groupe RPD3 / HDA1, régulait le temps de floraison en désacétylant et en réprimant l'expression du gène AGL19, et qu'il fonctionnait comme un régulateur négatif des gènes sensibles au stress. De plus, un autre membre des HDAC RPD3 / HDA1 de classe II, HDA15, a été présenté comme un module de répression clé dirigeant l'élongation de l'hypocotyle contrôlé par la lumière via la modulation de l'acétylation des histones dans différentes conditions de lumière. Dans mon travail de thèse, j'ai pu établir que les HDAC HDA19, HDA9 et HDA15 étaient

impliqués dans la thermomorphogenèse chez Arabidopsis. La perte fonctionnelle de HDA15 a conduit à un allongement constitutif de l'hypocotyle dans des conditions normales de température ambiante, alors que l'absence de HDA9 ou HDA19 a aboli la réponse à une élévation de la température, avec pour conséquence le développement d'hypocotyles plus courts que ceux des plantes de type sauvage. L'ensemble des analyses génétiques et moléculaires que j'ai conduites suggèrent en outre que HDA9 et HDA19 exercent des fonctions distinctes dans la régulation des réponses thermosensorielles. Les résultats suggèrent que bien qu'appartenant au même groupe, différents HDAC jouent des rôles distincts dans la thermomorphogenèse. Ces données fournissent également une nouvelle compréhension le façon dont les végétaux intègrent les signaux environnementaux via la machinerie épigénétique.

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université PARIS-SACLAY

ÉCOLE DOCTORALE
Sciences du végétal : du gène à l'écosystème

Titre : Fonction et régulation des histone-désacétylases en réponse au stress chez *Arabidopsis*

Mots clés : HDACs, réponse au stress, acide salicylique, température élevée

Résumé : L'acétylation/désacétylation des histones joue un rôle important dans la régulation de divers processus du développement des plantes et de leur réponse au stress. Par contre, la régulation de l'activité des histone-desacétylases (HDAC) par des signaux cellulaires et la relation fonctionnelle entre les différentes HDAC au cours de la réponse au stress oxydatif et d'une élévation de la température ambiante restent encore mal connus. Mon travail de thèse a comporté : 1) l'analyse de la modification post-traductionnelle de la protéine HDA19, régulée par redox et celle des conséquences sur la régulation de l'expression de gènes et la réponse à l'acide salicylique (SA) ; 2) l'étude fonctionelle de HDA9, HDA15 et HDA19 dans la réponse à une élévation de la température ambiante. Dans la première partie, nous montrons que le changement redox induit par SA régule l'accumulation nucléaire de la protéine HDA19 via une S-nitrosylation réversible. Le traitement à SA, ou au donneur physiologique d'oxyde nitrique, S-nitrosoglutathione, augmente les marques d'acétylation des histones d'HDA19 dans des plantules d'*Arabidopsis*. Des lignées mutantes d'*hda19* présentent un état plus oxydé avec une augmentation de l'expression de gènes associés au ROS/RNS, ainsi qu'une accumulation de nicotinamide adénine dinucléotide et de glutathionne. Ces résultats suggèrent que SA induit la S-nitrosylation d'HDA19, réduit son accumulation nucléaire et par conséquent augmente l'acétylation des histones.

Dans la seconde partie, nous montrons que HDA9, HDA19 et HDA15 sont toutes impliquées dans la réponse de la plante à l'élévation de la température ambiante. Des mutants *hda15* montrent une réponse constitutive à des températures élevées dans des conditions normales, alors que les mutants *hda19* et *hda9* ont des phénotypes insensibles à la température élevée. L'analyse de l'expression de gènes par RT-PCR et RNA-seq révèle que la mutation d'HDA15 provoque une augmentation de transcrits des gènes impliqués dans le métabolisme primaire et cellulaire lorsque les plantules sont transférées de 20°C à 27°C pendant 4 heures. Par contre, la mutation d'HDA19 conduit à l'induction de gènes impliqués dans des réponses au stress, alors que les gènes induits par la mutation d'HDA9 après le transfert à 27°C ne semblent pas concerner des catégories fonctionnelle spécifiques. Il semble donc que la réponse des plante à à l'élévation de la température soit régulées par HDA9 et HDA19 par différentes voies. Ces résultats suggèrent que de différents membres d'HDAC ont des rôles distincts ou opposés dans la réponse à l'élévation de la température, en affectant l'expression de gènes de différentes catégories. Les travaux de ma thèse apportent un éclairage nouveau sur la fonction des HDAC, en enrichissant la compréhension de la régulation de l'expression génique chez la plante.

Title : Function and regulation of *Arabidopsis* histone deacetylases in stress response

Keywords : HDACs, stress response, salicylic acid, warm temperature

Abstract : Histone acetylation/deacetylation play important roles in a diverse range of developmental processes and stress-responsive pathways in plants. However, little is known regarding the regulation of HDACs themselves by environmental signals, which may alter their function in the regulation of gene expression. Also HDACs functions in plant sensing of environmental conditions such as redox stresses and warm ambient temperature need to be precized. My thesis work focuses on: (1) The analysis of redox-regulated posttranslational modifications and their consequences on HDA19 function in gene regulation and in salicylic acid (SA)-mediated stress response; (2) The study of the function of HDA9, HDA15, and HDA19 in plant responses to warm temperature and thermal-related genes expression. In the first part, we show that SA-induced redox changes negatively regulate HDA19 nuclear accumulation through a reversible S-nitrosylation. Treatment with SA, as well as with the physiological nitric oxide donor S-nitrosoglutathione, increases the abundance of several histone acetylation marks of HDA19 in *Arabidopsis* seedlings. *hda19* mutant lines display a more oxidative status with increased ROS/RNS-related genes expression, as well as nicotinamide adenine dinucleotide and glutathione levels. These results suggest that SA affects histone acetylation by decreasing the nuclear accumulation of HDA19, resulting in histone hyperacetylation.

The second part of the study showed that HDA9, HDA15, and HDA19 are involved in modulating plant adaptation to higher ambient temperatures in *Arabidopsis*. Mutation of HDA15 displayed a constitutive warm temperature-responsive phenotype under normal temperature, whereas HDA9 and HDA19 mutants were shown insensitive to warming-temperature. Genes expression and RNA sequencing analysis revealed that HDA15 mutation led to the up-regulation of many genes involved in primary and cellular metabolic process when the seedlings were transferred from 20 °C to 27 °C for 4 h. On the other hand, *hda19* mutation resulted in up-regulation of genes mainly involved in stress-responses at both normal (20 °C) and warmer (27 °C) temperatures. However, up-regulated genes in *hda9-1* mutants did not appear enriched for any particular gene functional category when shifted from 20 °C to 27 °C. Likely, HDA9 and HDA19 positively regulate thermosensory elongation through distinct mechanisms. Our study suggested that the dynamics of histone acetylation regulated by HDA9, HDA15, and HDA19 plays an important role for plant adaptation to warm temperature, which involves distinct regulatory pathways of gene expression. Taken together, my thesis work brought in a few new elements to the current understanding of HDACs functions in the regulation of gene expression in plants.

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