Histone Deacetylase and Their Function in Plant Development

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Supported by the National Science Foundation Grant IOB0616096 to Z. L.
Abstract
Histone acetylation and deacetylation are directly connected with transcriptional activation and silencing in eukaryotes. Gene families for enzymes that accomplish these histone modifications show surprising complexity in domain organization, tissue-specific expression, and function. This review is focused on the family of histone deacetylases (HDACs) that remove the acetyl group from core histone tails, resulting in a “closed” chromatin and transcriptional repression. In *Arabidopsis*, 18 HDAC genes are divided into three different types, RPD3-like, HD-tuin, and Sirtuin, with 2 or more members in each type. The structural feature of each HDAC class, the expression profile of each HDAC gene during development, and functional insights of important family members are summarized here. It is clear that HDACs are an important class of global transcriptional regulators that play crucial roles in plant development, defense, and adaptation.

Key word: Histone deacetylase, Chromatin, Epigenetic Regulation, Plant Development, Gene Silencing
**Introduction**

Chromatin, consisting of both DNA and proteins, is responsible for storing heritable and instructional information in a cell. Chromatin is highly organized and consists of nucleosomes. In each nucleosome, four core histone proteins, H2A, H2B, H3, and H4, are organized into octameric protein complexes containing two molecules of each of the four core histones. ~146 base pairs of DNA wrap around each nucleosome, and ~80 base pairs of DNA link adjacent nucleosomes with the help of histone H1, forming the so-called "beads-on-a-string" organization. This basic level of chromatin packaging is further arranged into higher order conformations (Alberts et al. 2002). Protruding from the nucleosome are the positively charged amino-terminal tails of the core histone proteins that tightly associate with DNA’s negatively charged phosphate backbone. Reversible post-translational modifications of histone H3 and H4 amino-terminal tails, such as methylation, phosphorylation, ubiquitination, ADP-ribosylation and acetylation, alter interactions between the DNA and nucleosomes, resulting in changes in chromatin conformation. It was discovered that specific histone modifications at certain residues of the amino-terminal tails of H3 and H4 constitute the "Histone Code" that instructs the chromatin to adopt either "open" or "closed" configurations, thereby regulating the availability of cis-regulatory elements of genes to transcriptional machinery (Jenuwein and Allis 2001). Some of the histone modifications such as methylation are heritable. Therefore, the histone code not only expands the information-storing capacity of DNA but also offers rapid and reversible changes in chromatin accessibility when challenged with internal or external stresses. As plants are sessile, the ability to rapidly change their gene expression programs in response to internal or external stresses underlies the very plastic growth and developmental programs in plants.

Histone acetylation is a reversible process that plays vital roles in epigenetic regulation described above. Therefore, histone acetylation and deacetylation are of particular importance to plant growth, development, defense, and adaptation. Histone Acetyltransferase (HATs) and Histone Deacetylases (HDACs) are enzymes required to perform histone acetylation and deacetylation, respectively, acting on the ε-amino group of lysine residues located near the amino-termini of core histone proteins. The prime acetylation targets are H3, lysine (K) residues 9, 14,18, and 23, and H4 lysine (K) residues 8, 12, 16, and 20 (Fuchs et al. 2006). Although the lysine residues can only accommodate one acetyl group at a time, each nucleosome has over 20 possible targets.
for acetylation. The addition of acetyl groups, mediated by HATs, neutralizes the positive charge of histone tails and decreases their affinity for DNA. Growing evidence also indicates that acetylation may help shape the binding surface for activators and repressors (Kurdistani and Grunstein 2003). Thus acetylation allows the chromatin to open up and provides transcription factors and RNA polymerase access to the DNA (Mutskov et al. 1998; Puig et al. 1998). This is supported by much experimental data indicating that hyperacetylation of histone H3 and H4 is associated with transcriptionally active euchromatic regions. In contrast, hypoacetylation mediated by HDACs has an opposite effect on the chromatin, enabling the histones to bind more tightly to the negatively charged DNA. As a result, hypoacetylation is associated with the repression of gene expression (Hebbes et al. 1988; Chen and Pikaard 1997; Chua et al. 2001, 2003). In order to carry out their intended functions, HDACs and HATS interact with co-repressor, or co-activator complexes, respectively, to regulate the expression of target genes (Utley et al. 1998; Gonzalez et al. 2007).

In this review, we will focus on describing the roles histone deacetylases (HDACs) in Arabidopsis thaliana development. These genes are emerging as crucial players in all aspects of plant development including embryogenesis, abaxial/adaxial polarity determination, flowering and senescence as well as responses to day length and environmental stresses (Tian and Chen 2001; Devoto et al. 2002; He et al. 2003; Song et al. 2005; Xu et al. 2005; Zhou et al. 2005; Long et al. 2006; Benhamed et al. 2006; Ueno et al. 2007; Tanaka et al. 2008; Wu et al. 2008).

**Different HDAC Types**

The HDACs can be grouped into three types (Fig. 1; Table 1). The first type is homologous to the yeast RPD3 (Reduced Potassium Deficiency 3), which is present throughout eukaryotes and is most widely studied. The second type, the HD-tuins, appears to be present only in plants (Lusser et al. 1997; Wu et al. 2000b; Dangl et al. 2001). The structurally distinct third type is homologous to the yeast Sir2, which is a NAD-dependent enzyme.

The type I (RPD3-like) superfamily HDACs in Arabidopsis thaliana consist of 12 putative members (Pandey et al. 2002). All members have a characteristic histone deacetylase domain (Interpro: IPR003084) (Fig. 1). Based on sequence similarity, they are further divided into three
classes (Fig. 1; Table 1) (Pandey et al. 2002). Class I encompasses HDA19, HDA6, HDA7, and HDA9. Class II includes HDA5, HDA15, and HDA18. HDA2 and its two additional isoforms compromise class III. HDA8, HDA14, HDA10, and HDA17 are unclassified members of the RPD3-like superfamily, with both HDA10 and HDA17 bearing similarity to HDA9. There is a lot of structural diversity within this superfamily of proteins. In addition to the conserved HDAC domain, three RPD3 family members (HDA6, 7, and 9) have poly-glycine regions, five members (HDA6, 9, 15, 10, and 17) have aspartate-rich regions, one (HDA15) has a RanBP2-type zinc finger, and one (HDA18) has a coiled-coil domain (Fig. 1).

The type II (HD-tuins) HDACs are plant-specific HDACs originally identified in maize (Lusser et al., 1997). EST homology searches identified 4 Arabidopsis HD-tuins: HDT1, HDT2, HDT3, and HDT4 (Wu et al. 2000b; Dangl et al. 2001; Pandey et al. 2002). These proteins are structurally distinct from the RPD3 family and possess sequence similarity to the FKBP family peptidyl-prolyl cis-trans isomerase (Fig. 1) (Aravind 1998; Dangl et al. 2001). HD-tuins have a conserved amino terminal EFWG amino acid region, required for repression followed by a central acidic region rich in glutamic and/or aspartic acid. In HDT1, this acidic region is essential for catalytic activity, as its deletion resulted in compromised HDAC activity (Wu et al. 2000b). HDT1 and HDT3 contain a single C2H2 type zinc finger domain in the carboxyl terminus, which may enable high affinity DNA-binding or mediate protein-protein interactions (Aravind 1998; Wu et al. 2000b; Dangl et al. 2001; Zhou et al. 2004). However, for HDT1, the carboxyl terminal region including the zinc finger was shown unnecessary for transcription repression (Wu et al. 2000b).

The type III (Sirtuin) HDACs are based on their sequence homology to the yeast Silent Information Regulator 2 (Sir2) protein. They represent a unique group of NAD-dependent HDACs, which, unlike the Rpd3 and HD-tuin types, are not inhibited by Trichostatin A (TSA) or sodium butyrate (Jung 1997; Grozinger et al. 2001;). The sirtuins in all organisms are divided into five classes based on sequence motifs within their highly conserved Sir2 domain (Frye 2000; Imai et al. 2000). Arabidopsis has two sirtuin proteins, SRT1 and SRT2, belonging to classes IV and II, respectively. Interestingly, SRT2 has 5 or more alternate splice variants (Frye 2000; Pandey et al. 2002). Much needs to be learned about sirtuins in plants.
Expression of HDACs

Since all three types of HDACs consist of two or more family members, one important question is whether these family members play similar or distinct roles. We analyzed the mRNA expression profiles of 16 HDACs using the microarray data from AtGenExpress (Schmid et al. 2005), which examined mRNA expression in 79 diverse Arabidopsis tissues samples with the Arabidopsis ATH1 array. As shown in Fig. 2, the four HD-tuins family members (HDT1-HDT4) show highly similar expression profiles; all are highly expressed in inflorescences and young floral tissues but are under-expressed in vegetative tissues, pollens, seeds, and late stage flowers. With the exception of HDA7, the RPD3-class I HDAC genes (HDA6, HDA9, and HDA19) also exhibit similar expression profiles with high levels of expression in inflorescences and floral tissues and low levels of expression in vegetative tissues. In contrast, the two Sirtuin family members SRT1 and SRT2 exhibit very different expression profiles and may act in different tissues, stages, or processes. In addition, the RPD3-class III (HDA2) and RPD3-unclassified (HDA8 and HDA14) HDACs exhibited rather unique expression profiles with HDA14 the most unique among all HDACs, suggesting a highly distinct function of HDA14.

Functions of HDACs

RPD3-like HDAC: HDA19

Among all HDACS in Arabidopsis, HDA19 (also known as HD1 and RPD3A) is the most studied. HDA19 is expressed in all tissues throughout the life of the plant with high levels of expression in reproductive tissues (Fig. 2). Microarray data has revealed that over 7% of the genome is either up or down regulated in hda19 mutants, further illustrating the global role of HDA19 (Tian et al. 2005). Loss-of-function via antisense RNA and T-DNA insertion as well as overexpression via 35S::HDA19 studies support that HDA19 is a global regulator of gene expression in development as well as in responses to environmental stresses (Tian and Chen, 2001; Tian et al. 2003, 2005; Zhou et al. 2005). A wide range of developmental abnormalities was observed in these loss-of-function HDA19 lines. The mutant plants were shorter and flowers were abnormal (Fig. 3). hda19 flowers showed reduced numbers of petals, shorter stamen, reduced male and female fertility, and smaller siliques that often contain aborted seeds. Some
showed premature death of seedlings, asymmetrical development of the first two leaves, serrated and narrow leaves, and a prominent left handed twist of rosette leaves.

*hda19-1*, a T-DNA insertion line, is temperature-sensitive. At an elevated temperature (29 °C), seedlings developed disorganized root and shoot meristems with shoot meristems forming pin or tubular or single cotyledon phenotype. These phenotypes resemble those of the *Arabidopsis topless (tpl)* mutants (Long et al. 2006). Recent experiments illustrated a redundant role of *HDA19* and *HDA6* in the repression of embryonic program and embryogenesis-related genes after germination (Tanaka et al. 2008). In addition, 9% of 151 HDA19 antisense RNA lines experienced embryonic defects with many not surviving past two weeks (Tian and Chen 2001). Many of these early seedling abnormalities could result from ectopic expression of normally silenced genes, such as embryogenesis-related genes *LEAFY COTYLEDON1 (LEC1)* and *FUSCA3 (FUS3)* (Tanaka et al. 2008), precocious expression of floral-specific gene *SUPERMAN (SUP)*, and *NO APICAL MERISTEM (NAM)* (Tian and Chen 2001; Tian et al. 2003).

In addition to regulating development, *HDA19* also regulates plant's response to their environment. *Hda19* loss-of-function lines are slightly late flowering under long day (LD) conditions (Tian et al. 2003). While mutants of a histone acetyltransferase GCN5 showed long-hypocotyl phenotype and reduced expression of light-inducible genes, *hda19* mutants showed opposite effects. The double *gcn5; hda19* mutants were restored to normal. Chromatin immunoprecipitation experiments revealed that *gcn5* mutants exhibited reduced histone acetylation on the promoter regions of *CAB2, RBCS-1A*, and *IAA3*. *hda19* mutants, on the other hand, exhibited increased histone acetylation on the promoters of the same genes (Benhamed et al. 2006). Therefore, HDA19 works antagonistically with the GCN5 to regulate light-mediated processes.

Using HDA19 promoter-driven reporters, Zhou et al. (2005) showed that *HDA19* transcription is induced by treatment with Jasmonic acid (JA), ethylene, wounding, or pathogen *A. brassicicola* infection. Plants expressing 35S::*HDA19* not only exhibited generally decreased histone acetylation levels, but also an increased expression of Ethylene Response Factor1 (ERF1) as well as an enhanced resistance to pathogen *A. brassicicola*. Ethylene and JA regulated *PATHOGENESIS-RELATED (PR)* genes (basic chitinase and β-1,3-glucanase) were also
upregulated (Zhou et al. 2005). *HDA19-RNAi* plants showed opposite phenotypes and reduced expressions of corresponding downstream genes (Zhou et al. 2005). These data suggest that HDA19 regulates gene expression in the JA and ethylene signaling pathways in response to pathogens.

Histone deacetylases often act as part of larger protein complexes. Experimental evidence has linked HDA19 with several such complexes. HDA19 protein was shown to interact with the LEUNIG/SEUSS co-repressor complex, which, among other functions, is involved in the suppression of carpel and stamen identity in the outer two floral whorls by repressing *AGAMOUS* (Sridhar et al. 2004; Gonzalez et al. 2007; Liu and Karmarkar 2008). Interestingly, loss-of-function *hda19* mutants exhibited a floral phenotype similar to those of *leunig* (Tian and Chen 2001; Tian et al. 2003). *hda19* was shown to genetically interact with *topless* (*tpl*) co-repressor whose protein shares similar protein domains with the LEUNIG co-repressor. Hda19 mutants also exhibited a similar embryonic phenotype to *tpl-1* (Long et al. 2006).

The defects in acetylation and deacetylation are mostly reversible as phenotypic defects of *hda19* mutant plants were rescued in the heterozygous F1 progeny of a backcross to wild type. These F1 plants exhibited wild type levels of acetylation and no change in DNA methylation levels (Wu et al. 2000a; Tian and Chen 2001; Tian et al. 2003). Interestingly, *hda19/+* plants retain the same reduced levels of H3 lysine9 methylation as homozygous *hda19/hda19* plants (Tian et al. 2005).

**RPD3-like HDAC:HDA6**

*HDA6* gene is required for the silencing of transgenes, transposable elements, and ribosomal RNA (rRNA) genes (Murfett et al. 2001; Aufsatz et al. 2002; Lippman et al. 2003; Lawrence et al. 2004; Probst et al. 2004). Several *hda6* mutant alleles were identified in genetic screens designed to isolate mutants with increased expression of transgenes. Both post-transcriptional gene silencing (Murfett et al. 2001) and RNA-directed DNA methylation (Aufsatz et al. 2002) were thought to be involved. It was also shown that, like DNA methyltransferase (MET1) and chromatin remodeling ATPase (DDM1), HDA6 can silence the majority of the transposable element and may act in a similar pathway as MET1 and DDM1 (Lippman et al. 2003).
Most interestingly, HDA6 localizes, in part, to the nucleolus and HDA6 was shown to play a role in the epigenetic mechanism that underlies rRNA gene silencing in nucleolar dominance. Specifically, in *A. suecica*, the allotetraploid hybrid of *A. thaliana* and *A. arenosa*, the *A. thaliana*-derived rRNA genes are selectively silenced (Chen et al. 1998), a phenomenon called nucleolar dominance similar to the X-chromosome inactivation in mammals. In HDA6-RNAi knockdown lines, the silenced rRNA genes in the allotetraploid hybrid *Arabidopsis* are derepressed, so is the decondensation of the nucleolus organizer region (NOR), loss of DNA cytosine methylation at the rDNA promoter, and the loss of histone H3K9 dimethylation accompanied with the gain of H3K4 trimethylation, H3K9 acetylation, H3K14 acetylation, and histone H4 tetra-acetylation (Lawrence et al. 2004).

Given the highly similar gene expression profiles and sequence similarity between HDA6 and HDA19 (Fig. 2), it is not surprising that HDA6 and HDA19 regulate, perhaps redundantly, many of the same processes including suppression of embryonic program after germination, mediation of JA and ethylene signaling pathways, and promotion of flowering and senescence (Devoto et al. 2002; Zhou et al. 2005; Tanaka et al. 2008; Wu et al. 2008). In addition to decreased expression of JA responsive genes, PDF1.2, VSP2, JIN1, and ERF1 in *hda6* loss-of-function mutants or RNAi lines (Wu et al. 2008), HDA6 protein was found to interact with COI1, a F-box protein involved in JA signal perception or transduction (Devoto et al. 2002). F-box proteins are members of the SCF complexes that target specific proteins for ubiquitination and degradation. It is likely that The COI1-SCF complex regulates JA responsive genes by targeted ubiquitination and degradation of HDA6.

**Additional RPD3-like HDACs**

In *Arabidopsis*, single-layered root epidermal cells differentiate into hair and non-hair cells in a position-dependent manner. Treatment with trichostatin A (TSA), a specific inhibitor of histone deacetylase (HDAC), and *hda18* loss-of-function mutants showed increases in root hair density in the seedlings (Xu et al. 2005). Therefore, histone deacetylation may regulate key genes in root epidermal cell differentiation by, perhaps, mediating positional cues in roots.
HDA15 is unique among HDACs because it has a RanBP2-type zinc finger, which is known to associate with receptor-mediated transport between the nucleus and cytoplasm. Despite its unique domain, HDA15, like HDA19, HDT1, and HTD2, is involved in the assimilation of T-DNA in *Agrobacterium*-mediated transformations. HDA15-RNAi transgenic lines showed a "resistant to Agrobacterium transformation" phenotype (Crane and Gelvin 2007).

**Type II (HD-tuin) HDACs**

This is a plant-specific class of HDACs, which is of particular interest because HD-tuins may have evolved to perform plant-specific functions. With the exception of HDT4, the other three HD-tuins are characterized in further details. For example, HDT1, HDT2, and HDT3 were shown to repress transcription when they were fused to GAL4 DNA binding domain and tethered to reporter genes *in planta* (Wu et al. 2000b; Wu et al. 2003), and the amino-terminal EFWG and the histidine 25 (a potential catalytic residue) were shown to be important for the repressor activity (Zhou et al. 2004). *In situ* hybridization revealed that HDT1, HDT2, and HDT3 were all expressed in ovules, embryos, shoot apical meristem, and leaves. More interestingly, their expression was strongly induced during the process of somatic embryogenesis (Zhou et al. 2004). The *in situ* data is in general agreement with the microarray data (Fig. 2), which showed that all family members are expressed in inflorescences and young as well as old flowers. The highly similar mRNA expression profiles among the four HD-tuins (Fig. 2) suggest potential functional redundancy.

Consistent with its expression in ovules, embryos and during somatic embryogenesis, HDT1-silencing resulted in aborted seeds (Wu et al. 2000a). In contrast, 35S::GFP-HDT1 lines showed high frequencies of developmental abnormalities such as curved, narrow, or branching leaf phenotypes, flowers with shorter filaments, aborted seed development, sterility, and late flowering (Wu et al. 2000b; Zhou et al. 2004). This overexpression of HDT1 also resulted in the repression of genes associated with embryo development (Zhou et al. 2004).

Like HDA6, HDT1 plays a role in rRNA silencing. HDT1-RNAi resulted in the release of silencing of the Arabidopsis rRNA, an increase in histone H3K4 methylation, and loss of cytosine methylation at rDNA in *A. suecica*, the allotetraploid hybrid of *A. thaliana* and *A.*
arenosa (Lawrence et al. 2004).

HDT1 and HDT2 act in leaf polarity determination (Ueno et al. 2007). Specifically, HDT1-RNAi and HDT2-RNAi in asymmetric leaf 2 (as2) or as1 mutant background caused decreased adaxial gene PHABULOSA (PHB) expression and increased abaxial FILAMENTOUS FLOWER (FIL) expression, which correlated with the formation of abaxialized and filamentous leaves (Ueno et al. 2007). Further experimental data suggested that HDT1 and HDT2 could interact with AS1 or AS2 to regulate the generation or distribution of microRNA 165/166, which targets the PHB transcripts (Kidner and Martienssen 2004).

Recently, it was shown that HDT3 is involved in ABA stress response (Sridha and Wu 2006). HDT3 expression is repressed by ABA and over-expressing a HDT3-GFP transgene resulted in ABA insensitivity, reduced transpiration, and increased tolerance to salt and drought stress. Over-expression of HDT3 caused a upregulation of ABA response genes (Sridha and Wu 2006).

**Type III (Sirtuin) HDACs**

In yeast, Sir2 primarily deacetylates H4 lysine16, H3 lysine56, and H3 lysine9 and, to a lesser degree, H3 lysine14 (Imai et al. 2000; Xu et al. 2007). In addition to their histone deacetylation ability, yeast Sir2, and its bacterial and mammalian homologs, have NAD dependent ADP-ribosyl transferase activity, which function is distinct from its HDAC mediated activity (Frye 1999; Imai et al. 2000). Yeast Sir2 is primarily involved in the silencing of telomeres, rRNA, silent mating type loci, and the suppression of rDNA recombination (Rine and Herskowitz 1987; Gottlieb and Esposito 1989; Smith and Boeke 1997; Imai et al. 2000; Xu et al. 2007). Sir2 also has a role in cell longevity. It is involved in preventing the formation of the extra-chromosomal rDNA circles associated with cell aging. Sir2 promotes longevity in worms and flies, and mitotically active yeast lacking Sir2 have reduced life spans, while those with an extra copy live longer (Kaeberlein et al. 1999; Blander and Guarente 2004). However, Sir2 accelerates aging of cells grown in hypo-caloric media (Fabrizio et al. 2005).

In contrast to yeast, there is little experimental data available about plant Sirtuin HDACs. Treatment with sirtinol, an inhibitor of Sirtuin, inhibits body-axis formation and vascularization
in *Arabidopsis* seedlings (Grozinger et al. 2001); these phenotypes resemble the *monopterous* mutants defective in auxin signaling (Przemeck et al. 1996; Hardtke and Berleth 1998). Therefore, SRT1 and SRT2 may have a role in auxin signaling.

In rice, OsSRT1 is highly expressed in rapidly dividing cells, and RNAi knockdown induced DNA fragmentation and cell death, accompanied by an increased hydrogen peroxide production. This phenotype correlated with reduced H3 lysine 9 dimethylation and increase H3 lysine 9 acetylation in transposon and HR gene regions, suggesting a role for plant sirtuins in the HR response (Huang et al. 2007).

**Conclusion**

HDACs play both global and specific roles in gene regulation through their abilities to modify histones and change chromatin conformation. Much remains unknown as to the functional diversity and functional redundancy among different HDACs. One of the major challenges for plant biologists is the large number of HDAC genes, the possible large variety of HDAC-containing chromatin complexes, and the interactions among different transcription networks that utilize these HDACs. With the rapid development of new sequencing technologies as well as various tools for transcriptome and proteome analyses and modeling, the plant scientists are poised to make major contributions toward understanding the epigenetic regulatory mechanisms underlying the plasticity of plants.

**Acknowledgement**

We would like to thank Parsa Hosseini for analyzing the HDAC expression data shown in Fig. 2.
Reference


Gonzalez D, Bowen AJ, Carroll, TS, Conlan RS (2007). The transcription co-repressor LEUNIG interacts with the histone deacetylase HDA19 and mediator components MED14 (SWP) and CDK8 (HEN3) to repress transcription. Mol Cell Biol 27, 5306-5315.


A.

Class I

- HDA19
- HDA6
- HDA7
- HDA9

Class II

- HDA5

Class III

- HDA2*

Unclassified

- HDA14
- HDA10

B.

- HDT1
- HDT2
- HDT3
- HDT4

C.

- SRT1
- SRT2
Figure Legend

Figure 1. Domain organization of HDACs in *Arabidopsis thaliana*.

(A) Type I (RPD3-like superfamily) HDACs. Green boxes represent the conserved HDAC domain. Red regions are active sites necessary for histone deacetylase activity. *HDA2* isoform 2 is missing residues 268-387 and has a change at residue 253 from NRVYILDMY to SMIKTLYIS. *HDA2* isoform 3 is missing residues 208-235. HDA17 (At3G44490), which is similar to HDA9, is not shown.

(B) Type II (HD-tuins) HDACs. The red bar at the amino terminus represents the conserved EFWG region required for repression. The red box in the HDT1 represents an acidic region required for repression.

(C) Type III (Sirtuin) HDACs. Light blue boxes represent the conserved Sir2 domain. In all cases, G, D and E represent high glycine-, aspartate-, and glutamate-rich regions, respectively. CC represents a coiled-coil domain, and Zn represents a zinc finger domain. Domain organizations are drawn based on the UniProtKB (beta.uniprot.org).

Figure 2. Hierarchical cluster analysis of HDAC expression profile in different tissues and developmental stages.

HDAC gene names are indicated below each column; tissue type is indicated on the right. An increase in expression in a specific tissue is indicated by red, a decrease in expression is indicated by green, no change in expression is indicated by black (see the bar below the clustergram for specific fold change). The clustergram was generated with the Matlab RC13 (Mathworks) Bioinformatics Toolbox utilizing the data generated by AtGenExpress (development) (Schmid et al. 2005).

Figure 3. Representative phenotypes of *hda19* (Salk_139445) plants.

(A) A photo showing *hda19* (left) and wild type (right) plants. Both are in Col-0 background.

(B) A wild type flower.

(C) A flower from *hda19* plants growing at 20 °C.

(D) A flower from *hda19* plants growing at 29 °C. The more severe flower phenotype at 29 °C suggests that the *hda19* mutation is sensitive to high temperature.
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<thead>
<tr>
<th>Gene</th>
<th>Type</th>
<th>Localization and Expression</th>
<th>Function</th>
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<tr>
<td>HDA19</td>
<td>RPD3 Class I</td>
<td>Globally expressed with highest expression in reproductive tissues (Schmid et al., 2005).</td>
<td>Localizes in nucleus (but not nucleolus) (Fong et al., 2006; Long et al., 2006; Zhou et al., 2005). Global repressor involved in embryonic and flower development, JA and ethylene pathways, stress responses, light responses, and the assimilation of T-DNA (Grozinger et al., 2001; Huang et al., 2007).</td>
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<td>(At4G38130)</td>
<td>(Also HD1, RPD3A)</td>
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<tr>
<td>HDA6</td>
<td>RPD3 Class I</td>
<td>Globally expressed with highest level in reproductive tissues (Schmid et al., 2005).</td>
<td>Localizes in nucleus (Farkesy et al., 2006). Global repressor involved in regulating flowering, senescence, JA pathway, repression of embryonic fate, and establishment of nuclear dominance.</td>
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<tr>
<td></td>
<td>(At5G3110)</td>
<td>(Also AXE1, RPD3B, Sili)</td>
<td></td>
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<tr>
<td>HDA7</td>
<td>RPD3 Class I</td>
<td>Low level expression in all tissues except stage 9 flowers, which show a higher level of expression (Schmid et al., 2005).</td>
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