

# HD2-type histone deacetylases: Unique regulators of plant development and stress responses

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## Research Article

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# Abstract

Plants have developed sophisticated and complex epigenetic regulation-based mechanisms to maintain stable growth and development under diverse environmental conditions. Histone deacetylases (HDACs) are important epigenetic regulators in eukaryotes that are involved in the deacetylation of lysine residues of histone H3 and H4 proteins. Plants have developed a unique HDAC family, HD2, in addition to the RPD3 and Sir2 families, which are also present in other eukaryotes. HD2s are well conserved plant-specific HDACs, which were first identified as nucleolar phosphoproteins in maize. The HD2 family plays important roles not only in fundamental developmental processes, including seed germination, root and leaf development, floral transition, and seed development, but also in regulating plant responses to biotic and abiotic stresses. Some of the HD2 members coordinate each other to function. The HD2 family proteins also show functional association with RPD3-type HDACs and other transcription factors as a part of repression complexes in gene regulatory networks involved in environmental stress responses. This review aims to analyse and summarise recent research progress in the HD2 family, and to describe their role in plant growth and development and in response to different environmental stresses.

## Introduction

Plants, being sessile in nature, have developed sophisticated and complex mechanisms to respond to different developmental and environmental stress signals (Kapazoglou and Tsaftaris 2011). Epigenetic regulation plays an important role in various biological processes ranging from the maintenance of genome stability to developmental scheduling and regulating responses to various environmental stresses (Kim, et al. 2015; Luo, et al. 2012; Pokholok, et al. 2005; Wolffe 1998). Control of gene expression via epigenetic regulation is governed by DNA methylation and nucleosomal core histone modifications (Shinozaki and Yamaguchi-Shinozaki 2007; Urano, et al. 2010). A basic nucleosome structure consists of an octamer of core histone proteins, including H2A, H2B, H3, and H4 (Zhou, et al. 2013), with the N-terminal lysine residues (also called the histone tails) of histone H3 and histone H4 projected outward. These tails can be subjected to several types of post-translational modifications, including acetylation, methylation, phosphorylation, ubiquitination, and sumoylation. These histone modifications act as a switch to turn gene expression 'on' or 'off' and, therefore, provide a flexible method of regulating gene expression in response to developmental and environmental signals (Kouzarides 2007; Kurdistani, et al. 2004; Zhang and Reinberg 2001). Histone acetylation and deacetylation are important histone modifications and are catalysed by the enzymes called histone acetyltransferases (HATs) and histone deacetylases (HDACs), respectively. HATs are considered to be gene activators, as they transfer the acetyl group to lysine residues of core histone proteins, resulting in relaxed, transcriptionally active euchromatin and subsequent gene activation, whereas HDACs remove the acetyl groups from the lysine residues, resulting in a compact nucleosome structure, thereby leading to transcriptional repression of associated genes (Kuo, et al. 1996; Shahbazian and Grunstein 2007; Zhang, et al. 1998). HDACs and HATs often play roles as components of multiprotein and chromatin remodelling complexes (Ríos, et al. 2007; Saez, et al.

2008). Proper control of the acetylation status of histone lysine residues is crucial for the regulation of gene expression in eukaryotes (Pandey, et al. 2002; Pfluger and Wagner 2007).

HDACs are classified into three different families, including the Reduced Potassium Deficiency 3 (RPD3) family, the silent induced regulator 2 (SIR2) family, and the histone deacetylase 2 (HD2) family (Kim, et al. 2015; Luo, et al. 2012; Ma, et al. 2013; Pandey, et al. 2002). The former two families, RPD3 and SIR2, share sequence homology with yeast HDACs and are found in different eukaryotes, whereas the HD2 family shares no sequence homology to yeast HDACs and has only been identified in plants and green algae (Bourque, et al. 2016; Ma, et al. 2013). This plant-specific HD2 family is emerging as a crucial player in different aspects of plant growth and development and in response to different environmental stresses. This review will update the current knowledge of the HD2 family with special attention to its regulation in plant development and response to environmental stresses.

## Evolution And Molecular Characteristics Of The Plant-specific Hd2 Family

The plant-specific HD2 family is thought to have evolved through multiple rounds of progressive genome duplication; this corresponds to the presence of at least two HD2 genes in each of the dicots (Bourque, et al. 2016). Based on phylogenetic analysis of DNA and protein sequences of HD2s, Pandey, et al. (2002) suggested that a single HD2 gene in the ancestors of monocots and dicots led to the development of all other HD2 genes in each of the monocot and dicot species. For example, Arabidopsis contains four HD2 family members, *HD2A*, *HD2B*, *HD2C*, and *HD2D*, which resulted from three successive rounds of genome duplication events (Bourque, et al. 2016; Soltis, et al. 2009). Sequence homology analysis of Arabidopsis HD2s based on DNA and protein sequences shows that the fourth member, *HD2D*, is a distantly related member of the HD2 family (Han, et al. 2016). The phylogenetic tree indicates the largest genetic distance between *HD2D* and other HD2 members, while *HD2A* and *HD2B* show the highest sequence homology (**Figure 1**).

HD2s were first identified as nucleolar phosphoproteins in maize, where expression of HD2-type genes was associated with actively dividing embryonic cells (Lusser, et al. 1997). The HD2 family was later identified in various plant species, with numbers varying in different plant species. Four members were identified in Arabidopsis and maize, six in soybean and tobacco, two in rice and barley, and three in tomato (Bourque, et al. 2016; Dangl, et al. 2001; Demetriou, et al. 2009; Hollender and Liu 2008; Nicolas-Francès, et al. 2018; Wu, et al. 2003; Yang, et al. 2018; Zhao, et al. 2015).

Based on phylogenetic analysis of at least 1205 HD2 sequences, Bourque, et al. (2016) reported that the first four amino acids of the pentapeptide motif (MEFWG) in the N-terminal domain of HD2 proteins are fully conserved, whereas the fifth residue (G) is conserved in 96% of the sequences analysed. The pentapeptide MEFWG motif and histidine 25, surrounded by hydrophobic amino acids in the N-terminal domain, are fully conserved in Arabidopsis, maize, soybean, tobacco, and rice. The presence of these conserved residues along with aspartate/glutamate at position 69 is considered to be important for

catalytic activity in gene-regulation mechanisms (Bourque, et al. 2016; Dangl, et al. 2001; Zhou, et al. 2004). The length of almost all of the HD2s ranged from 232 to 384 amino acids. The differences in the lengths of HD2s are associated with the length of the highly variable central acidic domain (Bourque, et al. 2016). Although HD2 proteins are regarded as gene repressors that act via deacetylation activity, the deacetylase domain of HD2 family proteins does not significantly match with any of the other HDAC family proteins, suggesting a specific role of the HD2 family in the biological processes in plants. The C-terminal domain of most HD2 proteins contains a putative C<sub>2</sub>H<sub>2</sub>-type (CxxC(x)<sub>12</sub>H(x)<sub>4</sub>H) zinc-finger (ZnF) motif, a unique structural feature of the plant-specific HD2 family (**Figure 2**). The nature and locus of these ZnF residues suggest that they belong to the TFIIIA class of ZnF. ZnF is generally considered to be associated with DNA-protein interactions because of the presence of the conserved motif, QALGGH. Because HD2 proteins lack this conserved motif and appear to have no other DNA-binding motifs, it is very likely that ZnF is associated with protein-protein interactions in gene regulatory networks (Bourque, et al. 2016; Dangl, et al. 2001; Mackay and Crossley 1998; Takatsuji 1998). The presence or absence of ZnF residue in HD2 proteins could possibly play an important role in implicating functional diversity, as suggested by Nicolas-Francès, et al. (2018).

## Biological Functions Of Hd2s In Plant Growth And Development

**HD2 family in Arabidopsis:** Plants undergo various physiological and molecular changes to respond to different internal and external signals (Liu, et al. 2014; Nakashima, et al. 2009). Members of the HD2 family play a significant role in plant developmental processes. In Arabidopsis, *HD2A*, *HD2B*, and *HD2C* genes are predominantly expressed in primary leaves, embryonic tissues, flowers and siliques whereas the expression of *HD2D*, a distantly related HD2 family member, is observed mainly in stem and floral parts, with the lowest expression observed in the leaves and roots (Wu, et al. 2000; Wu, et al. 2003; Zhou, et al. 2004). Different expression patterns of *HD2D* indicate that this gene may have different functions compared to other HD2 genes in Arabidopsis.

HD2s are involved in many plant development-related processes including vegetative growth, flowering and seed development in Arabidopsis (see **Figure 3 and Table 1**). *HD2A* plays a role in the development of inflorescence stems in Arabidopsis. The *hd2a* mutants exhibited reduced height and thickness of the inflorescence stem. Transmission electron microscopy revealed that the size of individual fibre cells and tracheary elements was significantly decreased with increased cell wall thickness in the stem of *hd2a* mutants (Zhang, et al. 2019). The expression of many secondary wall-related biosynthetic genes, including lignin and cellulose, was affected in the mutants, indicating the role of *HD2A* in the development of vascular tissues in the stem of Arabidopsis. Antisense silencing of *HD2A* in Arabidopsis results in aborted seed development with reduced siliques, whereas overexpression of *HD2A* causes delayed flowering and poor seed development. Expression of several seed development-responsible genes, including *LEA*, *ABF3*, and *SMP*, is repressed in the overexpression lines (Wu, et al. 2000; Wu, et al. 2003; Zhou, et al. 2004).

Vegetative growth, including root, shoot, and leaf development, is strongly associated with the modification of histone acetylation. A significant decrease in Arabidopsis primary root growth was observed in *hd2a/hd2b* double mutants compared to *hd2a* and *hd2b* single mutants. Quantitative PCR analysis showed that the expression of HD2B was significantly increased in the *hd2a* single mutant line. This shows an additive effect of *HD2A* and *HD2B* in controlling root growth in single mutants (Li, et al. 2017). The findings further showed that *HD2B* binds to the promoter region of GIBBERELLIN 2-OXIDASE2 (*GA2ox2*). Hyperacetylation of the lysine 9 residue of histone 3 (H3K9) at the locus of *GA2ox2* was found in both the *hd2b* and *hd2a/hd2b* mutants. *GA2ox2* overexpression lines showed the same decrease in root length as the double mutants, whereas knockout of *GA2ox2* in the *hd2a/hd2b* double mutant line rescued root growth compared to wild-type (WT) (Li, et al. 2017). This indicates that *HD2A* and *HD2B* coordinate to modulate the *GA2ox2* pathway to regulate root growth in Arabidopsis.

Using genome-wide association mapping and transcriptome analysis, Yano, et al. (2013) identified *HD2B* as a genetic factor related to seed dormancy in Arabidopsis. Expression of *HD2B* is significantly upregulated (> 10 folds) in seeds under cold and after ripening (dry storage of seeds), treatments which are adopted to break seed dormancy, suggesting that downregulation of *HD2B* may be required to maintain seed dormancy. Gibberellins (GA) are required in breaking seed dormancy and stimulating seed germination. Gibberellins level was significantly increased in *HD2B* transgenic lines. Transgenic seeds treated with an HDAC inhibitor, trichostatin A (TSA), showed reduced accumulation of endogenous GA in imbibed seeds. This indicates the role of *HD2B* in seed germination rather than seed dormancy (Colville, et al. 2011; Yano, et al. 2013). These reports do not precisely depict the role of *HD2B* in seed dormancy and germination, and further research is required in this regard.

Arabidopsis ASYMMETRIC LEAVES 1 and 2 (*AS1*, *AS2*) genes control leaf morphology. RNA interference (RNAi)-mediated simultaneous knockdown of *HD2A* and *HD2B* in an *as2* mutant background caused an abnormal distribution of microRNA165/166 in leaves and an abaxial filamentous leaf phenotype. Further studies indicated that both *HD2A* and *HD2B* interact with *AS1* and/or *AS2* to mediate the expression and/or distribution of miRNA165/66 (Kidner and Martienssen 2004; Ueno, et al. 2007), suggesting that *HD2A* and *HD2B* coordinate with *AS1* and *AS2* to play functional roles in establishing leaf morphology in Arabidopsis. The HD2s may coordinate with each other and with other proteins to play roles in mediating the expression of target genes to regulate plant developmental processes.

Flowering induction is specifically dependent on day length in Arabidopsis. FLOWERING LOCUS T (*FT*) is a florigen gene that promotes flowering in Arabidopsis under long-day conditions. Guo, et al. (2020) studied the epigenetic regulation mechanism of the *FT* gene in Arabidopsis and reported that *HD2C* is recruited to the locus of *FT* at dusk via an *MRG1/2* dependent pathway to regulate flowering time under long-day conditions. *HD2C* binds with histone methylation readers *MRG1/2* and deacetylates lysine 9, 23, 27 of histone H3 to repress *FT* gene expression and early flowering.

Colville, et al. (2011) studied the role of HD2 genes during germination and early stages of seedling growth. The *hd2a* mutants were found to show increased germination, whereas *hd2c* mutants

showed decreased germination on glucose-containing germination medium. However, *HD2A/hd2c* double mutants showed germination similar to that of WT. This suggests an interesting antagonistic function of *HD2A* and *HD2C* in regulating germination, thus fine-tuning the germination in double mutants.

*Arabidopsis* transgenic plants overexpressing *HD2D* showed delayed germination, decreased primary root length with more lateral roots, and a high root to shoot ratio compared to WT plants (Farhi, et al. 2017; Han, et al. 2016). *HD2D* transgenic plants also showed a significant delay in flowering (Han, et al. 2016), whereas *hd2d* mutants showed an early flowering phenotype under both long- and short-day conditions (Farhi, et al. 2017). Several flowering inducer genes, including *SOC1*, *FUL*, *SEP3*, and *FT*, were downregulated in *HD2D* transgenic plants, suggesting a role for the *HD2D* gene in regulating the network genes in plant transition from vegetative to reproductive stage. It is still not clear whether *HD2C* and *HD2D* coordinate to target a subset of genes involved in early flowering. The underlying mechanism of *HD2A* and *HD2B* in plant flowering is yet to be elucidated. These studies suggest that HD2s may work in a coordinated fashion to play multiple roles in regulating plant growth and development in *Arabidopsis*.

**HD2 family in rice:** Besides the model plant *Arabidopsis*, HD2s have also been studied in some crops, including rice, potato, and tomato (Zhao, et al. 2015; Zhao, et al. 2015). Studies in rice have demonstrated the involvement of HD2s in plant development and flowering time. Overexpression of *HDT701* triggered early flowering in hybrid rice under long-day conditions compared to its parents (*Oryza sativa* ssp. *indica* and *O. sativa* ssp. *japonica*), which was probably owing to the resulting suppression of the over-dominant flowering time repressor gene Heading date 1 (*Hd1*), leading to early flowering. The expression of several other flowering-related genes was also affected in hybrid rice (Li, et al. 2011). Another study reported that *hdt701* rice mutants showed delayed flowering under both short-day and long-day conditions. Expression of the flowering suppressor genes *Hd1* and *IDS1*, an upstream repressor of *Ehd1*, was significantly increased in *hdt701* mutant lines. The ChIP assay revealed that *HDT701* binds to *IDS1* in the promoter region to inhibit its expression, leading to flowering induction (Cho, et al. 2018). Knockdown of *HDT702* also caused abnormal plant height with narrow leaves and stems (Hu, et al. 2009). These data indicate that HD2s play important roles in regulating flowering time and plant development in rice.

**HD2 family in potato and tomato:** *ScHD2a*, an ortholog of *Arabidopsis HD2A* in potato (*Solanum chacoense*), showed elevated accumulation of transcripts in ovules after fertilisation (Lagacé, et al. 2003), suggesting that this gene is important for normal seed development. RNAi-mediated silencing of the HD2-type *SIHDT3* gene in tomato resulted in delayed fruit ripening and prolonged shelf life. RNAi-*SIHDT3* plants showed lower ethylene content and reduced expression of ripening-associated genes, including *E4*, *E8*, *RIN*, *Pti4*, and *LOXB*. This finding suggests that the HD2-type *SIHDT3* plays a role in the ethylene biosynthetic pathway and delays fruit ripening (Guo, et al. 2017).

**HD2 family in woody plants:** The role of the HD2 family in woody plants has not yet been widely studied. Recently, a study was performed on poplar (*Populus trichocarpa*) plants to investigate the functions of the HD2 family (Ma, et al. 2020; Tong, et al. 2018). Researchers cloned the HD2-type genes, *PtHDT902* and *PtHDT903*, and investigated their expression, localisation, and functions in poplar. Both proteins were

found to be localised to the nucleus. Overexpression of *PtHDT902* in poplar and Arabidopsis caused increased gibberellin biosynthesis, with increased primary root length in Arabidopsis. Transgenic poplar showed hypersensitivity to salt stress with limited adventitious root formation. Decreased transcript levels of salt responsive genes including HIGH-AFFINITY K<sup>+</sup> TRANSPORTER 1 (*HKT1*) and GALACTINOL SYNTHASE 4 (*Go/S4*) were observed in the transgenic poplar lines (Ma, et al. 2020). This indicates that *PtHDT902* plays a negative role in salt stress response in poplar. However, the specific role of HD2 in herbaceous and woody plants may be different and will, therefore, require further study.

## Role As Nucleolar Phosphoproteins In Ribosomal Genes Regulation

As typical nucleolar localising proteins, HD2-type HDACs are considered to play a role in the regulation of ribosomal genes (Kim, et al. 2014; Lawrence, et al. 2004; Lusser, et al. 1997). Lawrence, et al. (2004) studied the nucleolar dominance in *A. suecica*, an allotetraploid hybrid of *A. thaliana* and *A. arenosa*. The hybrid contains transcriptionally silenced rRNA genes inherited from *A. thaliana* and transcriptionally active rRNA genes inherited from *A. arenosa* (Chen, et al. 1998). The *A. suecica* hybrid treated with TSA (HDAC inhibitor) showed re-activation of silenced *A. thaliana*-inherited rRNA genes and significantly upregulated the expression of *A. arenosa*-inherited rRNA genes. Interestingly, RNAi-mediated knockdown of *HD2A* in *A. suecica* resulted in the loss of H3K9 deacetylation, accompanied by loss of H3K9 dimethylation and de-repression of the rRNA genes inherited from *A. thaliana*. The nucleolar localisation of *HD2A* indicates that this HDAC may play a direct role in the rRNA gene silencing required for nucleolar dominance (Lawrence, et al. 2004). Another report published by the same group in 2007 demonstrated that rRNA gene silencing-based nucleolar dominance was progressively established at the early postembryonic tissue developmental stage. RNAi-mediated knockdown of *HD2A* and *HDA6* in *A. suecica* disrupted the condensation and repression of developmentally regulated nucleolus organiser regions (NORs) of *A. thaliana*-derived rRNA genes along with a decreased connection to histone 3, which is dimethylated at lysine 9 (H3K9me<sub>2</sub>) (Pontes, et al. 2007). This finding suggests that both *HDA6* and *HD2A* are necessary for establishing nucleolar dominance at the early stages of vegetative development. According to Pontes, et al. (2007), both proteins localise to the nucleolus, which indicates that these proteins may interact and act directly on the target rRNA genes in the establishment of postembryonic nucleolar dominance in *A. suecica*.

Chen, et al. (2018) demonstrated that both *HD2B* and *HD2C* co-localise in the nucleolus, while *HD2C* interacts with *HD2B* and forms homo- and/or hetero-dimers. Genome-wide analysis revealed that *HD2C* interacts and represses the expression of important ribosome biogenesis-related genes in the nucleolus. Loss of function single or double mutants of *HD2B* and *HD2C* displayed abnormal accumulation of 18S pre-rRNA intermediates, further resulting in an aberrant developmental pleiotropy, involving short roots and narrow leaves (Chen, et al. 2018). Ribosomal protein S6 (RPS6), which is also localised in the nucleolus, interacts with *HD2B* and plays a possible role in the transcriptional regulation of rRNA genes in Arabidopsis. Overexpression of both genes together in protoplasts resulted in a decreased accumulation of 18S pre-rRNA intermediates, triggering downregulation of some rRNA genes (Kim, et al. 2014),

suggesting that these genes may play a collective and direct role in ribosome biogenesis and ultimately in plant development.

## Biological Functions Of Hd2 Family Members In Response To Environmental Stresses

The homeostatic external environment is a key factor regulating plant growth, reproduction, and survival. Plants facing an imbalanced external environment undergo a series of morphological, physiological, and molecular changes during growth (Mehrotra, et al. 2014; Sah, et al. 2016). In plants, gene regulatory networks related to environmental stress responses have been studied by analysing the different stress-responsive genes that are responsible for the synthesis of functional and regulatory proteins such as transcription factors (Fujita, et al. 2011; Sah, et al. 2016). Modifications in the histone acetylation status of stress-responsive genes caused by biotic and abiotic stresses are important underlying mechanisms of gene expression regulation, which have been investigated in many plant species. Environmental stresses can cause changes in the acetylation status of lysine 9 and 14 (K9, K14) at histone H3 and lysine 18 and 27 (K18, K27) at histone H4 of stress-responsive genes (Chen, et al. 2010; Kim, et al. 2015; Kuang, et al. 2012; Luo, et al. 2012; Song, et al. 2020), indicating that a large group of functionally related genes are regulated in a coordinated manner through histone modifications in different stress responses in plants. Yeast and animal-based studies have demonstrated that HDACs often play roles as components of multiprotein and chromatin remodelling complexes (Ríos, et al. 2007; Saez, et al. 2008).

Plants can withstand environmental stresses by modifying their behaviour and stimulating the production of stress hormones. The phytohormone, abscisic acid (ABA), plays an important role in regulating various developmental processes and adaptive stress responses, and is produced in increased quantities by plants upon stimulation (Fujita, et al. 2011). ABA is thought to regulate nearly 10% of protein-coding genes in *Arabidopsis*. The expression of ABA-responsive genes is regulated by a number of ABA-related transcription factors (Fujita, et al. 2011). Many ABA-dependent and independent transcription factors are known to be regulated by HDACs (Glass and Rosenfeld 2000; Jepsen and Rosenfeld 2002). Reports have shown the involvement of RPD3-type HDACs in regulating plant responses to abiotic stresses in different species via ABA-dependent pathways (Kuang, et al. 2012; Song, et al. 2019). This section summarizes the recent research progresses in the HD2 family in relation to their role in response to internal and external cues in different species.

**Arabidopsis:** HD2 family genes show differential expression patterns in response to various abiotic stresses (Ding, et al. 2012; Grandperret, et al. 2014; Hollender and Liu 2008; Kuang, et al. 2012; Sridha and Wu 2006; Wu, et al. 2003). Cold treatment of *Arabidopsis* plants caused the upregulation of many HDAC genes, including HD2-type *HD2A*, *HD2B*, and *HD2C* genes. Most of these genes showed gradual upregulation, indicating a corresponding response to cold stress (To, et al. 2011). Contrary to cold response, expression of all HD2s was repressed by salt and ABA (Kuang, et al. 2012; Luo, et al. 2012).

*HOS15*, a DDB1-CUL4 Associated factor (DCAF) protein, encodes one of 85 WD40-repeat proteins in Arabidopsis which directly interact with specific targets by acting as a substrate receptor for its ubiquitination and subsequent degradation by the proteasome (Ali and Yun 2020; Fonseca and Rubio 2019). Zhu, et al. (2008) reported the association of *HOS15* with *HD2C* in histone modification in response to cold stress in Arabidopsis. Study demonstrated that *HOS15* acts as a DCAF protein and interacts with and degrades the *HD2C* protein via ubiquitination and proteasome-mediated degradation, thus modulating the expression of cold-responsive (COR) genes under freezing stress in Arabidopsis (Park, et al. 2018). Lim, et al. (2020) studied the function of the *HOS15* binding protein, POWERDRESS (*PWR*) in response to cold stress. This study showed that *HOS15* interacts with *HD2C* with the help of *PWR* at the promoter region of COR genes, thus mediating the degradation of *HD2C*, leading to the upregulation of COR genes as a part of the cold stress response. The *pwr* mutants showed down-regulation of the COR genes with increased sensitivity to cold stress. This suggests that these three proteins associate together to build a histone-modifying complex of *HOS15-PWR-HD2C* and regulate the expression of COR genes in cold stress response.

Buszewicz, et al. (2016) revealed *HD2C* as a positive regulator of heat stress response. Upon heat exposure, *HD2C* transcript levels were significantly upregulated, which downregulated the expression of heat-responsive genes. The *hd2c* and *brm* (a subunit of the *SWI/SNF* complex) knockout mutants displayed growth-retarding phenotypes upon heat exposure for 24 h (Buszewicz, et al. 2016). Transgenic plants overexpressing *HD2C* displayed an insensitive phenotype to exogenous applied ABA and showed tolerance to salt and drought stresses with decreased transpiration rates. Expression of ABA-responsive genes, including LATE EMBRYOGENESIS ABUNDANT PROTEIN (LEA)-class genes, *RD29B* and *RAB18*, was upregulated in *HD2C* overexpression lines (Kuang, et al. 2012; Sridha and Wu 2006). Kuang, et al. (2012) showed that *HD2C* interacts with the RPD3-type *HDA6* protein and regulates plant responses to ABA and salt stresses. The level of gene activation mark histone H3K9K14ac in the promoter region of ABA-responsive genes, *ABI1* and *ABI2*, was found to be higher in *hda6/hd2c* double mutant plants. The loss-of-function *hd2c* T-DNA insertion mutants displayed increased sensitivity to ABA during germination and showed decreased tolerance to abiotic stresses compared to WT (Kuang, et al. 2012). These reports support the hypothesis that *HD2C* is involved in ABA pathways in mediating the plant stress response. Later, using a bimolecular fluorescence complementation assay, it was shown that *HD2A* and *HD2D* can also physically interact with *HDA6*. HD2 proteins were also shown to interact with *HDA19* (Kuang, et al. 2012a; Luo, et al. 2012b). Overexpression of *HD2D* in Arabidopsis also displayed enhanced tolerance to salt, drought and cold stress (Han, et al. 2016). Currently, it is not clear whether *HD2C* and *HD2D* coordinate to respond to these abiotic stresses. Both ABA and other stresses can induce changes in histone acetylation levels of some ABA and abiotic stress-responsive genes, suggesting that HDACs and some transcription factors work in co-ordination to regulate functionally related genes in abiotic stress responses. Further research is needed to reveal whether the induction of different HD2s are linked to each other and how ABA works in coordination with HD2s to mediate the expression of target genes in response to different abiotic stresses.

Buszewicz, et al. (2016) performed GFP-binding affinity chromatography to precipitate interacting proteins of *HD2C* and found that *HD2C* was bound to *HD2A* and *BRM*-containing *SWI/SNF* chromatin remodelling complexes. This suggests the functional association of *HD2C* with *HD2A*, indicating that some HD2s may work together in a close relationship with gene regulation activity. Functional association within HD2 family members as well as with RPD3-type HDACs as part of repression complexes may be critical for regulating gene expression through histone modifications.

*Arabidopsis* DNA METHYL TRANSFERASE 2 (*AtDNMT2*) has been found to be involved in regulating gene expression (Cao and Jacobsen 2002). *DNMT2*, which is responsible for the methylation of DNA or RNA in *Arabidopsis*, is dependent on the activity of the HD2 family. A region of the N-terminal domain of *AtDNMT2* showed physical interaction with *HD2A*, *HD2B*, and *HD2C*, but not with *HD2D*. A significant decrease in the repression activity of *AtDNMT2* was observed in the *hd2c* mutant line (Song, et al. 2010). Since HD2s are involved in ABA and abiotic stress responses, the relationship between *DNMT2* and HD2s indicates an interplay between DNA methyltransferases and histone deacetylases in response to abiotic stresses.

Regarding biotic stress response in *Arabidopsis*, pathogen-associated molecular patterns (PAMPs), also called microbial-associated molecular patterns (MAMPs) are major regulators of plant innate immunity against plant pathogens and introduce mega-scale changes in gene expression patterns (Asai, et al. 2002; Ausubel 2005). *MPK3* is a plant mitogen-activated protein (MAP) kinase, activated during PAMP-triggered MAPK signalling pathways upon recognition of plant pathogens (Gao, et al. 2008; Meng and Zhang 2013). It is believed that PAMPs initiate systematic acquired resistance (SAR) against a broad spectrum of pathogens in plants by inducing localised programmed cell death (PCD), as a hypersensitive response (HR) to confine the pathogenic infection (Ma and Berkowitz 2007). Latrasse, et al. (2017) reported that the MAP kinase *MPK3* directly interacts with and phosphorylates *HD2B* during PAMP-triggered immunity, where *MPK3* directly employs *HD2B* in the reprogramming of defence-related gene expression by modulating acetylation of lysine 9 of histone 3 (H3K9) to mediate innate immunity in *Arabidopsis*. They also reported that *HD2B* relocates to the nucleus from the nucleolus upon recognition of bacterial flagellin fragment flg22, thus inducing global H3K9 histone deacetylation to mediate the expression of bacterial defence-related genes in *Arabidopsis*.

**Rice and barley:** Overexpression of *HDT701* in rice displayed decreased acetylation levels at H4K5K16 (histone H4, lysine K5, and K16), and showed hypersensitivity to different rice pathogens, including *Magnaporthe oryzae* and *Xanthomonas oryzae*. Knockdown of *HDT701* showed an opposite response with increased expression of defence-related genes and PAMPs, as well as hyposensitivity to rice pathogens, suggesting *HDT701* as a negative regulator of innate immunity. Using the ChIP assay, Ding, et al. (2012) also found that *HDT701* can directly interact with a subset of defence pathway genes including *MAPK5* and *MAPK6*, and transcription factors such as *WRKY71* and *WRKY53* to regulate their expression. This suggests that *HDT701* downregulates defence-related genes, thus negatively regulating plant innate immunity. Although, studies on the involvement of the HD2 family in biotic stress responses are limited, studies on *Arabidopsis* and rice provide evidences that the HD2 family is involved in the biotic stress

responses, and functions by coordinating with defence pathway-related genes. Further research on different species are required to further validate these finding.

*HDT701* showed stable expression throughout the plant life cycle. *HDT701* transgenic rice plants showed hypersensitivity to salt, drought and ABA stress during germination, however, and hyposensitivity to these stresses at the seedling stage (Zhao, et al. 2015). In contrast, *hdt701* mutant seedlings displayed increased sensitivity to both salt and drought stresses (Wai and An 2018). These mutants showed a dramatic increase in expression levels of *WRKY45*, an upstream regulator of the ABA-related genes, *SNAC1* and *NCED4*. Expression of the HD2-type genes; *HDAC2-1* and *HDAC2-2* in barley and *HDT701* and *HDT702* in rice, was significantly upregulated upon exogenous application of jasmonic acid (JA) and salicylic acid (SA), whereas rice HD2 gene expression was decreased in response to ABA exposure (Demetriou, et al. 2009; Fu, et al. 2007). Barley HD2-type genes showed a divergent response to ABA treatment. These finding suggests that the rice HD2 gene, *HDT701*, may be involved in ABA-related pathways to mediate plant stress response.

**Tobacco:** Bourque, et al. (2011) studied the tobacco HD2-type proteins *NtHD2A* and *NtHD2B*. Exposure of tobacco plant to cryptogein, an elicitor family protein, secreted by oomycete *Phytophthora cryptogea* caused the phosphorylation of both HD2-type HDACs. The expression of these two genes was found to decrease significantly upon exposure to cryptogein, suggesting that cryptogein is an inhibitor of HD2s, thus leading to cryptogein-induced programmed cell death in tobacco (Bourque, et al. 2011). HD2-type proteins containing the ZnF motif in Arabidopsis (*AtHD2A/ AtHD2C*) and tobacco (*NtHD2A/ NtHD2B*) showed similar germination responses to salt stress compared to non-ZnF HD2 proteins (*NtHD2C, AtHD2B/ AtHD2D*) (Nicolas-Francès, et al. 2018; Sridha and Wu 2006), indicating that HD2 proteins containing ZnF motifs may have functional redundancy compared to non-ZnF HD2 proteins.

**Longan fruit:** Kuang, et al. (2012) demonstrated that the HD2-type gene, *DiHD2*, from longan fruit interacts with the ETHYLENE-RESPONSIVE FACTOR 1 (*DIERF1*) transcription repressor and regulates the expression of genes involved in longan fruit senescence. The *DiHD2* and *DIERF1* genes showed upregulation of their transcript levels in relation to longan fruit senescence stored under different temperature conditions. *PtHDT902* overexpressing poplar plants demonstrated hypersensitivity to salt stress. Expression of *PtHDT903* was downregulated under salt stress and upregulated under cold stress (Ma, et al. 2020; Tong, et al. 2018). These studies show the versatile functionality that the HD2 family plays in different species in different aspects of plant development and response to environment stress.

HD2s show functional diversity in terms of their role in plant developmental processes and biotic and abiotic stress responses. HD2 family members may not be redundant proteins and can be functionally coordinative or complementary. However, these HD2s may follow different pathways to accomplish common functions. Taken together, these studies provide significant evidence that the HD2 family plays a role in different biological processes to address the plant's internal and external challenges.

## Concluding Remarks

It is well established that the modification of histone acetylation levels plays an important role in plant development and response to environmental stresses. The existence of another HDAC family in addition to the RPD3 and SIR2 families put forward an interesting question as to why evolution provided the plants with the third plant specific HD2 family. HDACs, in general, are recruited to target areas to modify the chromatin structure at the given loci to inhibit gene transcription. HD2, being a unique HDAC family in plants, plays critical roles not only in fundamental developmental processes, including seed germination, root and leaf development, floral transition, and seed development, but also in regulating plant responses against biotic and abiotic stresses (**Table 1**). Members of the HD2 family have shown functional associations with RPD3-type HDACs, and many other genes as part of repression complexes in gene regulatory networks (**Figure 4**). Studies in mutants and overexpression lines of HD2C and HD2D have shown that these HD2s show similar responses towards different abiotic stresses, suggesting that HD2s may follow different pathways to accomplish the same functions. HD2s, which are involved in plant developmental processes and stress responses, show functional diversity, indicating that these may not be redundant proteins, but are functionally either independent or coordinative and/or complement. Unravelling the upstream signalling pathways that are responsible for the induction and biological functions of the HD2 family and downstream target genes and gene regulatory networks offers an important perspective, and could contribute to the understanding of the mechanisms by which the HD2 family regulates plant developmental processes and stress responses.

## Declarations

### Author Contributions:

MT and LT conceived the idea and planned the manuscript. MT performed the literature search and data analysis and wrote the manuscript. MT and LT critically edited the manuscript.

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### Conflict of interests:

Authors declare no conflict of interests.

## Tables

**Table 1:** Main features of characterized plant-specific HD2 proteins in different species

Species	Name	Accession	AA*	Protein domains	Putative targets	Functions
<i>Arabidopsis thaliana</i>	<i>HD2A</i> ( <i>HDT1</i> )	AT3G44750	245	HDAC, NLS, ZnF	miRNA165/miRNA166, <i>GA2ox2</i> rRNA genes, Seed development-related genes	Establishing the leaf polarity and nucleolar dominance, role in germination and seed and root development (Lawrence, et al. 2004; Li, et al. 2017; Ueno, et al. 2007; Wu, et al. 2000; Zhou, et al. 2004)
	<i>HD2B</i> ( <i>HDT2</i> )	AT5G22650	306	HDAC, NLS	miRNA165/miRNA166, <i>GA2ox2</i> , rRNA genes	Establishing the leaf polarity and nucleolar dominance, role in germination, root development and stress tolerance (Chen, et al. 2018; Kim, et al. 2014; Li, et al. 2017; Ueno, et al. 2007; Wu, et al. 2000; Yano, et al. 2013; Zhou, et al. 2004)
	<i>HD2C</i> ( <i>HDT3</i> )	AT5G03740	294	HDAC, NLS, ZnF	<i>ABI1, ABI2, RD29B,</i> <i>RAB18</i> <i>APX2, HSP101, HSP70T</i> <i>HSFA3,</i> <i>COR15A, FT</i>	Role in ABA and abiotic stress responses (salt, heat, cold), role in germination and flowering, establishing nucleolar dominance (Buszewicz, et al. 2016; Chen, et al. 2018; Luo, et al. 2012; Sridha and Wu 2006; Zhu, et al. 2008)
	<i>HD2D</i> ( <i>HDT4</i> )	AT2G27840	203	HDAC, NLS	<i>SOC1, FUL, SEP3, FT</i>	Role in floral transition, root development and abiotic stress responses (Farhi, et al. 2017; Han, et al. 2016)
<i>Oryza sativa</i> (Rice)	<i>HDT701</i>	Os05g51830	297	HDAC, NLS, ZnF	<i>WRKY45, Hd1, IDS1,</i> Defense-related genes	Role in flowering, germination, role in plant hormones and stress response (Ding, et al. 2012; Li, et al. 2011; Zhao, et al. 2015)
	<i>HDT702</i>	Os01g68104	274	HDAC, NLS, ZnF		Role in germination and normal plant development, role in plant hormones and stress response (Hu, et al. 2009; Zhao, et al. 2015)
<i>Solanum chacoense</i> (Wild Potato)	<i>ScHD2a</i>	Q6V9I6	269	HDAC, NLS, ZnF		Function during fertilization and seed development (Lagacé, et al. 2003)
<i>Dinocarpus longan</i> (Longan fruit)	<i>DiHD2</i>		305	HDAC, NLS, ZnF		Role in fruit senescence (Kuang, et al. 2012)

<i>Solanum lycopersicum</i> (Tomato)	<i>SIHDT3</i>	NM_001365033	306	HDAC, NLS, ZnF	<i>E4, E8, RIN, Pti4, LOXB</i>	Role in fruit ripening and shelf life (Guo, et al. 2017).
<i>Populus trichocarpa</i> (Poplar)	<i>PtHDT</i> 902	XP_006384671	276	HDAC, NLS, ZnF	<i>HKT1, GolS4</i>	Negatively regulate root formation and salt stress response by affecting stress related genes (Ma, et al. 2020; Tong, et al. 2018).

\* AA represents amino acids. Empty boxes in the table represents unavailability of data or information for the given gene and specie.

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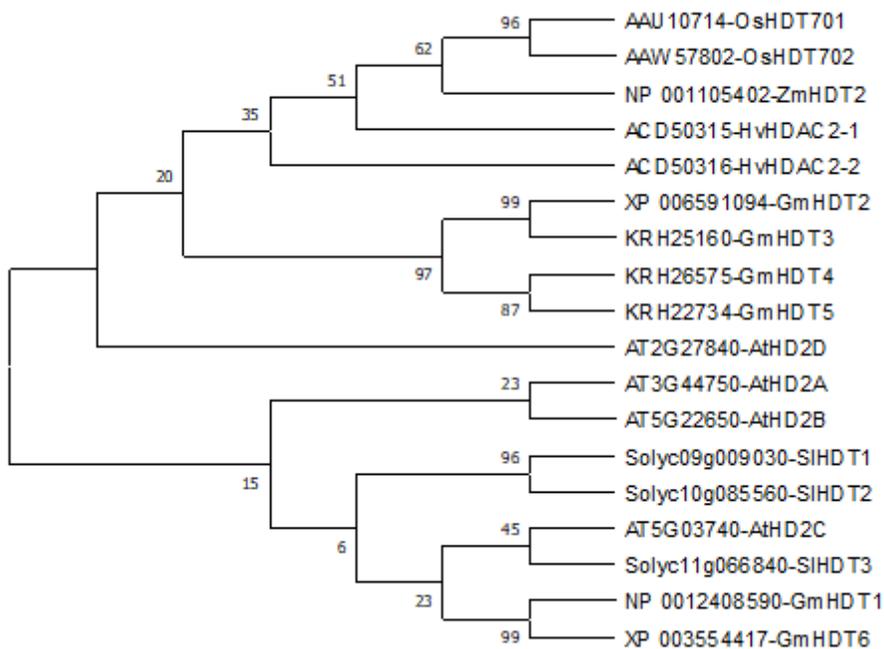
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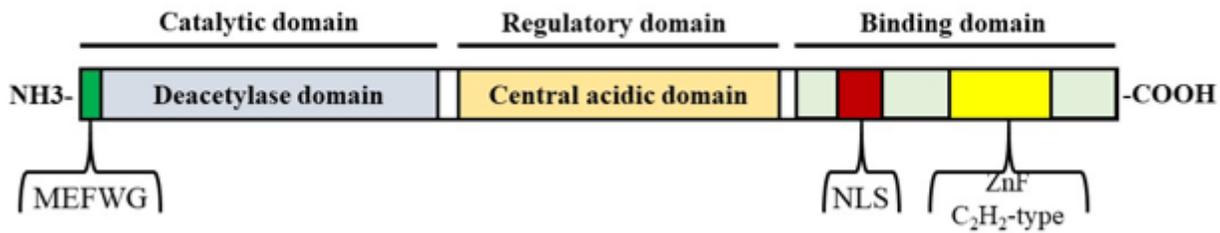
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## Figures



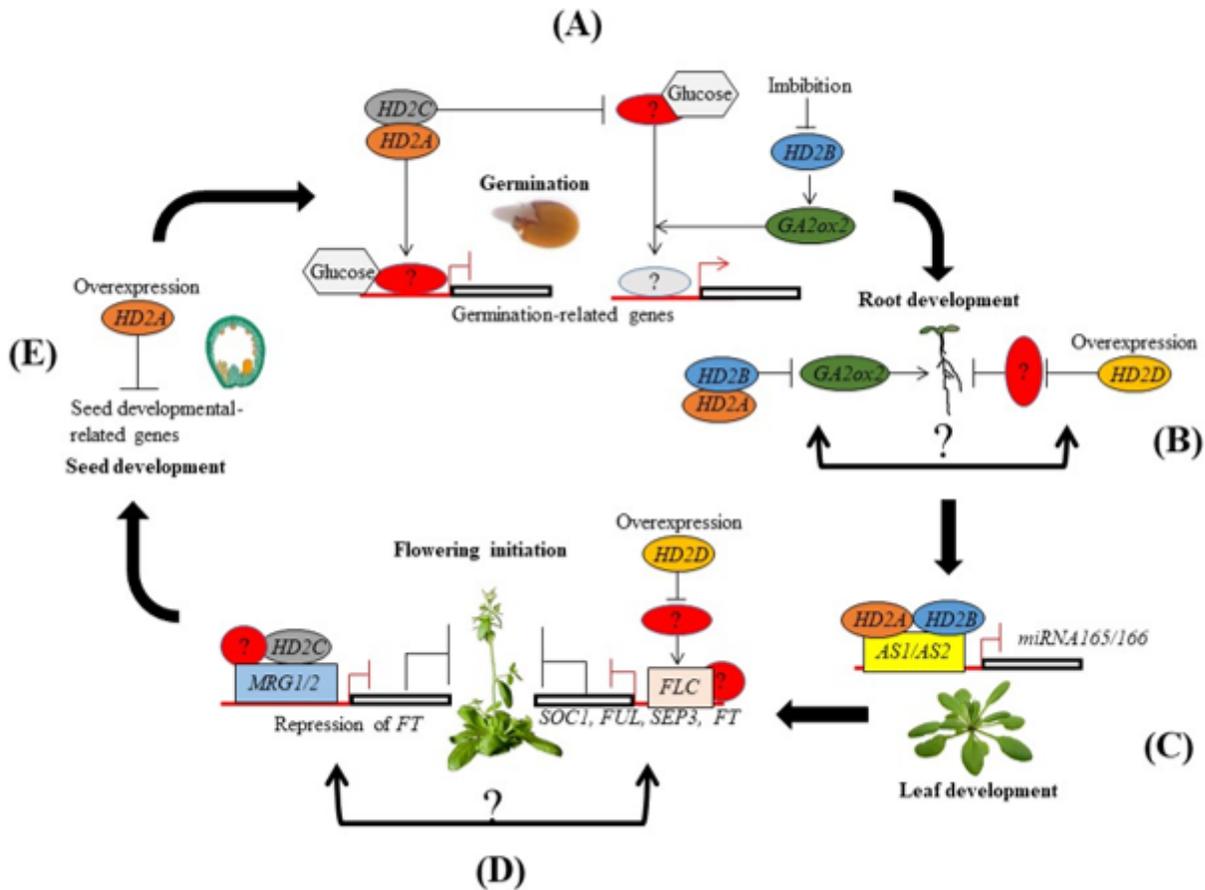
**Figure 1**

Phylogenetic analysis of HD2 family proteins in plants. Protein sequences of HD2s from *A. thaliana*, *Glycine max* (soybean), *O. sativa* (rice), *S. lycopersicum* (tomato), *Hordeum vulgare* (barley), and *Zea mays* (maize) were obtained from National Center for Biotechnology Information (<https://www.ncbi.nlm.nih.gov/>). Phylogenetic tree was constructed by Maximum Likelihood method (with bootstrap analysis of 1000 replicates and partial deletion parameters) using MEGA X software. The numbers at the nodes indicate the bootstrap values. Each name includes an accession number followed by a gene name.



**Figure 2**

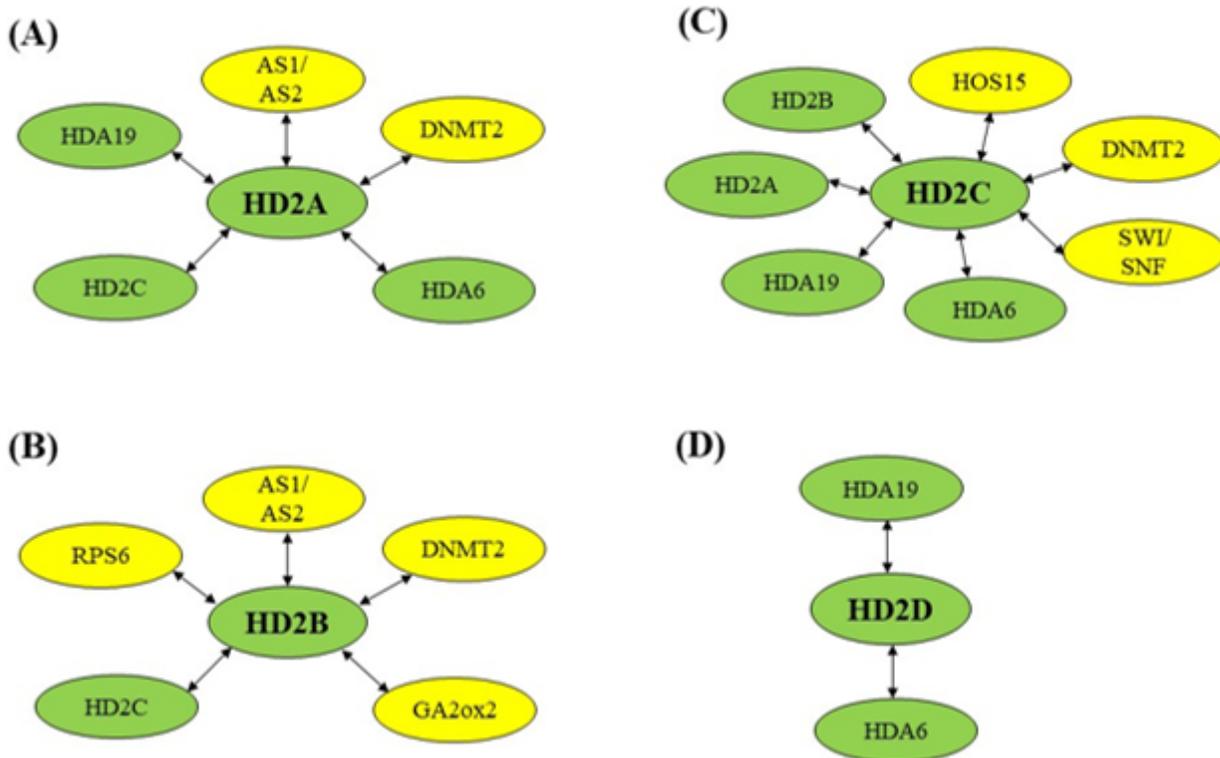
Schematic representation of plant-specific HD2 protein domains. This is a typical representation of Arabidopsis HD2 protein. Green box indicates a conserved region (MEFWG) in N-terminal domains of all HD2-type proteins, Red box represents Nuclear localization signal (NLS) and yellow box contains a putative C2H2-type ZnF motif.



**Figure 3**

The working model of HD2-type HDACs involved in different stages of Arabidopsis plant growth and development. (A) Role of HD2 family genes in germination: HD2A and HD2C work antagonistically to regulate the expression of germination related genes in the glucose-based germination pathway. HD2B regulates the expression of GA2ox2 (GA) which plays a role in seed germination. (B) Role of HD2 family genes in root development: HD2A and HD2B coordinate to repress the expression of GA2ox2 which

negatively regulates the root development. HD2D is negative regulator of root development. (C) Role of HD2 family genes in leaf development: HD2A and HD2B interact with AS1/2 and repress the miRNA165/166 for normal leaf polarity and development. (D) Role of HD2 family genes in flowering initiation: HD2C interact with MRG1/2 resulting in the repression of FT gene leading to repress flowering initiation. Overexpression of HD2D repressed the flowering time related genes in the downstream of signaling pathway resulting in delayed flowering. (E) Role of HD2 family genes in seed development: HD2A is involved in regulation of seed developmental related genes in Arabidopsis. All arrow heads represent positive regulators, while all stop lines represent negative regulators associated with related pathways. Black rectangles indicate open reading frames (ORFs). Reports have shown functional redundancy of HD2C and HD2D in regulating flowering time (D, as indicated by black double arrowhead bracket with question mark). However, there is no direct evidence that these HD2 coordinate with each other or follow different pathways to accomplish similar function.



**Figure 4**

Relation and network of HD2 family proteins in Arabidopsis based on current knowledge. HD2 proteins associated with other HDACs (green) and non-HDAC proteins (yellow) are involved in plant growth and stress response in Arabidopsis. Double head arrows between HD2s and other proteins indicate physical interaction. (A) Physical interaction of HD2A with three HDAC proteins (including HD2C of HD2 family) and two non-HDAC proteins. (B) Physical interaction of HD2B with one HDAC protein (HD2C) of HD2 family and four non-HDAC proteins. (C) Physical interaction of HD2C with four HDAC proteins (including HD2A and HD2B of HD2 family) of and three non-HDAC proteins. (D) Physical interaction of HD2D with two HDAC proteins (belonging to RPD3 family).