

Identification of HDA15-PIF1 as a key repression module directing the transcriptional network of seed germination in the dark

Dachuan Gu^{1,2,†}, Chia-Yang Chen^{3,†}, Minglei Zhao¹, Linmao Zhao^{1,2}, Xuewu Duan⁴, Jun Duan^{4,*}, Keqiang Wu^{3,*} and Xuncheng Liu^{1,*}

¹Key Laboratory of South China Agricultural Plant Molecular Analysis and Genetic Improvement, South China Botanical Garden, Chinese Academy of Sciences, Guangzhou 510650, China, ²University of Chinese Academy of Sciences, Chinese Academy of Sciences, Beijing 100049, China, ³Institute of Plant Biology, National Taiwan University, Taipei 106, Taiwan and ⁴Guangdong Provincial Key Laboratory of Applied Botany, South China Botanical Garden, Chinese Academy of Sciences, Guangzhou 510650, China

Received January 18, 2016; Revised April 7, 2017; Editorial Decision April 10, 2017; Accepted April 11, 2017

ABSTRACT

Light is a major external factor in regulating seed germination. Photoreceptor phytochrome B (PHYB) plays a predominant role in promoting seed germination in the initial phase after imbibition, partially by repressing phytochrome-interacting factor1 (PIF1). However, the mechanism underlying the PHYB-PIF1-mediated transcription regulation remains largely unclear. Here, we identified that histone deacetylase15 (HDA15) is a negative component of PHYB-dependent seed germination. Overexpression of *HDA15* in *Arabidopsis* inhibits PHYB-dependent seed germination, whereas loss of function of *HDA15* increases PHYB-dependent seed germination. Genetic evidence indicated that HDA15 acts downstream of PHYB and represses seed germination dependent on PIF1. Furthermore, HDA15 interacts with PIF1 both *in vitro* and *in vivo*. Genome-wide transcriptome analysis revealed that HDA15 and PIF1 co-regulate the transcription of the light-responsive genes involved in multiple hormonal signaling pathways and cellular processes in germinating seeds in the dark. In addition, PIF1 recruits HDA15 to the promoter regions of target genes and represses their expression by decreasing the histone H3 acetylation levels in the dark. Taken together, our analysis uncovered the role of histone deacetylation in the light-regulated seed germination process and identified that HDA15-PIF1 acts

as a key repression module directing the transcription network of seed germination.

INTRODUCTION

Seed germination is a crucial process in the seed plant life cycle, determining when plants enter natural or agricultural ecosystems. In *Arabidopsis*, mature seeds consist of an outer layer of dead tissues, the testa, underneath which the endosperm single-cell layer surrounds the embryo. Seed germination involves the sequential phases of testa rupture followed by endosperm rupture and radicle elongation, cotyledon expansion and greening (1,2). A wide range of environmental factors affect seed germination, including temperature, moisture, light and nutrient availability (1).

Light is a crucial environmental factor regulating seed germination, which is perceived by photoreceptors, such as phytochromes, cryptochromes, phototropins and UVR8 (3,4). Phytochromes are the major photoreceptors that promote seed germination in various plant species (5,6). Phytochromes are synthesized in a signaling inactive state known as Pr. Upon red light absorption, Pr converts to the signaling active form Pfr. In *Arabidopsis*, five phytochromes (PHYA-PHYE) were identified which regulate shared but distinct light responses (7). PHYA promotes seed germination in response to the very low fluence response (VLFR) and the far-red high irradiance response, while PHYB promotes seed germination in response to the red light low fluence response (LFR) (8). Since PHYA is expressed only after prolonged imbibition, PHYB plays a predominant role in the initial phase of seed germination (6,9).

Phytochromes regulate various light responses by initiating the transcriptional cascades of 10–30% of the en-

*To whom correspondence should be addressed. Tel: +86-20-37083652; Fax: +86-20-37083652; Email: xunchengliu@scbg.ac.cn
Correspondence may also be addressed to Jun Duan. Tel: +86-20-37252978; Fax: +86-20-37252978; Email: duanj@scib.ac.cn
Correspondence may also be addressed to Keqiang Wu. Tel: +886-2-33664546; Fax: +886-2-33663738; Email: kewu@ntu.edu

[†] These authors contributed equally to the paper as first authors.

tire transcriptome (10,11). Further studies showed that PHYB mediates light-regulated transcription and seed germination partly by destabilizing its interacting protein, phytochrome-interacting factor1 (PIF1) (12,13). PIF1 is a basic helix-loop-helix (bHLH) protein, which functions as a repressor of seed germination and is degraded via ubiquitin-26S proteasome upon interacting with the Pfr form of PHYB (13,14). The specific function of PIF1 in seed germination is partly due to the fact that it is highly expressed in imbibed seeds compared to other PIFs, such as PIF3, PIF4 and PIF5 (15). PIF1 represses seed germination partially by indirectly inhibiting the gibberellic acid (GA) pathway while activating the ABA pathway (12,16). Genome-wide transcription analyses indicated that PIF1 directly regulates genes involved in hormone signaling and cell wall modification in repression of seed germination (17,18). These findings suggested that PIF1 plays a crucial role in the light-initiated seed germination process. However, the mechanism underlying the PIF1-directed transcription regulation keeps largely unclear.

Gene transcription can be activated or repressed by post-translational histone modifications, including histone acetylation, methylation, phosphorylation and ubiquitination (19). Acetylation and deacetylation of lysine residues in the N-terminus of core histones are catalyzed by histone acetyltransferases and histone deacetylases (HDACs), respectively. The reversible histone acetylation and deacetylation provide a flexible mechanism for regulation of gene expression (20,21). In *Arabidopsis*, HDACs are grouped into three families, RPD3/HDA1 (Reduced Potassium Dependence 3/Histone Deacetylase 1), SIR2 (Silent Information Regulator 2) and HD2 (Histone Deacetylase 2) (22). Loss of function of the RPD3/HDA1-type HDAC *HDA19* in *Arabidopsis* seedlings results in a shorter hypocotyl phenotype and increased light-responsive gene expression, whereas the mutation of *HDA15* increases chlorophyll biosynthetic and photosynthetic gene expression (23,24). Genome-wide histone modifications analysis revealed that the activation of photosynthetic genes correlates with dynamic acetylation of H3K9 and H3K27 in response to light (25). These studies suggested an involvement of HDACs in light-regulated gene expression. Application of Trichostatin-A, an HDAC inhibitor, leads to inhibited seed germination of maize (*Zea mays*) and *Arabidopsis thaliana* (26,27). Further studies displayed that loss of function of *HDA9* in *Arabidopsis* induces reduced seed dormancy and faster germination compared with wild-type (28). These findings revealed a possible role of HDACs in control of seed germination. However, the mechanism of HDAC-mediated transcription regulation in light-initiated seed germination has not been reported.

In the present study, we identified that HDA15 is a negative regulator of light-initiated seed germination. HDA15 and PIF1 interact and co-repress the transcription of the light-responsive genes involved in multiple hormonal signaling pathways and cellular processes. Furthermore, HDA15 and PIF1 co-target to the genes involved in light-dependent seed germination and decrease the histone H3 acetylation levels of these genes in imbibed seeds. Our studies uncovered the role of histone deacetylation in light-regulated seed germination process and identified that

HDA15-PIF1 acts as a key repressive module directing the transcriptional network of seed germination-related genes.

MATERIALS AND METHODS

Data access

The datasets reported in this study have been submitted to the NCBI Gene Expression Omnibus (GEO; <http://www.ncbi.nlm.nih.gov/geo/>) under accession number GSE96848.

Plant materials and growth conditions

The seeds used in this study are all in Col-0 ecotype background. *hda15* mutant *hda15-1* (23) and *pif1* mutant *pif1-1* (13) were obtained from the *Arabidopsis* Information Resource Center (<http://www.arabidopsis.org/>). *35S:TAP-PIF1* seeds was a kind gift from professor Enamul Huq at the University of Texas. *35S:HDA15-GFP* and *35S:HDA15-TAPs* transgenic plants were generated as reported previously (23). For *PIF1:PIF1-GFP* transgenic lines, the promoter and full length cDNA of *PIF1* was subcloned into pCambia-1302 vector and the 35S promoter of vector was removed. The primers used for the construct were listed in Supplementary Table S1. The *PIF1:PIF1-GFP* construct was then transformed into *pif1* plants using the floral dip method (29). *Arabidopsis* plants were grown under long-day conditions (16 h WL/8 h dark) at 22°C. Following seed harvesting, seeds were dried in an incubator at 22°C for about 1 month prior to germination assays.

Germination assays

The PHYB-dependent seed germination assays were performed as described previously (16). Briefly, seeds were surface-sterilized and plated on half-strength Murashige-Skoog (Sigma-Aldrich) agar plates containing 0.3% sucrose and 1% phytoagar (pH 5.7). The plates were placed in an illuminated incubator with white light ($80 \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$) at 22°C. One hour after sterilization, seeds were irradiated with far-red light ($3.82 \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$) for 5 min (indicated as FR or dark conditions), or exposure to far-red light ($3.82 \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$) for 5 min following irradiation with red light ($13.12 \mu\text{mol} \cdot \text{m}^{-2} \cdot \text{s}^{-1}$) for 5 min (referred as FR/R or light conditions). The seeds were kept in the dark either for 12 h for the gene expression and ChIP analyses, or kept in the dark for 5 days to calculate the germination rates. At least 60 seeds were used for each experimental point, and three biological replicates were used for statistical analysis.

Yeast two-hybrid assays

Yeast two-hybrid assays were performed according to the Matchmaker GAL4-based two-hybrid system 3 protocol (Clontech). The full length or deletion versions of *HDA15* and *PIF1* cDNAs were subcloned into pGADT7-AD and pGBKT7-BD vectors. The primers used for the constructs were listed in Supplementary Table S1. The paired AD and BD constructs were co-transformed into the yeast strain AH109 using the lithium acetate method and plated on Minimal Media Double Dropouts (SD-Leu/-Trp). The co-transformants were then transferred onto Minimal Media

Quadruple Dropouts (SD/-Leu/-Trp/-Ade/-His) supplemented with 3-amino-1, 2, 4-triazole (3-AT) to test for possible interactions.

Bimolecular fluorescence complementation (BiFC) assays

Full-length cDNAs of *HDA15* and *PIF1* were subcloned into the pCR8/GW/TOPO vectors and then recombined into the YN (pEarleyGate201-YN) and YC (pEarleyGate202-YC) vectors (30). The primers used for the constructs were listed in Supplementary Table S1. The YN and YC vectors were transformed into *Agrobacterium tumefaciens* strain (GV3101) separately, and then co-infiltrated with the leaves of 2- to 4-week-old tobacco (*Nicotiana benthamiana*) plants. The transfected plants were kept in the dark for 2 days and the transfected epidermal cells were imaged using a TCS SP5 confocal spectral microscope imaging system (Leica).

In vitro pull-down assays

In vitro pull-down assays were performed as described (23). Full length cDNAs of *HDA15* and *PIF1* were subcloned into pGEX-4T-3 and pET32a vectors, respectively. The primers used for the constructs were listed in Supplementary Table S1. His-PIF1 recombinant protein was incubated with 30 ml His resin (QIAGEN) in a phosphate buffer (10 mM Na₂HPO₄, 10 mM NaH₂PO₄, 500 mM NaCl and 10 mM imidazole) for 2 h at 4°C, the binding reaction was washed three times with the phosphate buffer and then HDA15-GST or GST was added and incubated for an additional 2 h at 4°C. After washing three times with the phosphate buffer, the pulled-down proteins were eluted by boiling, separated by 10% sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) and detected by western blotting using an anti-His antibody.

Co-immunoprecipitation assay (Co-IP) assays

After FR or FR/R irradiation, the seeds of Col-0 and *TAP-PIF1* were kept in the dark for 12 h, the protein was then extracted with extraction buffer (50 mM Tris-HCl at pH 7.4, 150 mM NaCl, 2 mM MgCl₂, 1 mM DTT, 20% glycerol, 1% NP-40, 2 mM PMSF and 0.05 mM MG132) containing protease inhibitor cocktail (Roche). An anti-HDA15 antibody was added to the protein extracts for immunoprecipitation for 4 h, then 50 µl SureBeads™ protein A magnetic beads (Catalog no 161-4011, Bio-Rad) were added and incubated for 6 h. The beads were collected and washed for two times with a wash buffer (50 mM Tris-HCl at pH 7.4, 150 mM NaCl, 2 mM MgCl₂, 1 mM DTT, 10% glycerol, 1% NP-40, 2 mM PMSF). The immunoprecipitated proteins were then detected by SDS-PAGE gel with an anti-myc antibody (Catalog no M4439, Sigma). Endogenous HDA15 protein was detected with anti-HDA15 antibody, and PIF1-TAP protein was detected with anti-myc antibody.

qRT-PCR assays

After FR or FR/R treatment, the seeds were incubated in the dark at 22°C for 12 h. Total RNA was extracted

with TRIZOL Reagent (Invitrogen) according to the manufacturer's protocol. After DNase I treatment, the first strand cDNA was synthesized using 2 µg total RNA according to the manufacturer's instruction of TransScript One-Step gDNA Removal and cDNA Synthesis Super Mix Kit (TransGen, Beijing). Real-time polymerase chain reaction (PCR) was performed by using SYBR Green Mix (Bio-Rad) in an ABI7500 Real-Time PCR System (Applied Biosystems). All qRT-PCR experiments were independently performed in triplicate, and representative results were shown. *PP2A* was used as an internal control. The primer pairs for quantitative RT-PCR are listed in Supplementary Table S1.

RNA-seq analysis

For whole genomic transcriptome analysis, total RNA was extracted as described as above and an mRNA-seq library was prepared by using an mRNA Seq Kit (Illumina). RNA-seq were performed by Millennium Genomics with triplicate biological samples. High-quality clean reads were obtained by removing the adaptor sequences, ambiguous reads ('N' > 10%), and low-quality reads (i.e. more than 50% of bases in a read had a quality value Q ≤ 5). Then the clean reads were mapped to Arabidopsis genome TAIR10 using HISAT2 software with default parameters (31). Cuffdiff (<http://cufflinks.cbc.umd.edu/manual.html>) was applied to detect differentially expressed genes. Genes with more than 1.5-fold changes with statistically significance (*P*-value < 0.05) were selected. Gene ontology (GO) slim analysis was performed by using BINGO (www.psb.ugent.be/cbd/papers) (32) with default parameters. Functional classification was performed by using the DAVID functional annotation clustering tool with highest classification stringency (<http://david.abcc.ncifcrf.gov/home.jsp>) (33).

ChIP assays

ChIP assays were performed as previous described (34) with minor modifications. The seeds were cross-linked with 1% formaldehyde solution under a vacuum for 1 h. The chromatin was extracted and sheared to an average length of 500 bp by sonication, and then immunoprecipitated with specific antibodies including anti-GFP (Catalog no 6795, Sigma) and anti-acetyl-histone H3 (Catalog no 06-599, Millipore). The cross-linking was then reversed and the amount of each immunoprecipitated DNA fragment was determined by quantitative PCR using gene specific primers (Supplementary Table S1).

RESULTS

HDA15 is a negative component in PHYB-mediated seed germination

Previous reports indicated that PHYB plays a major role in light-initiated seed germination (6). To test whether HDA15 acts in the PHYB-mediated seed germination pathway, we analyzed the germination rates of a *hda15* knockout mutant (*hda15-1*) and two *HDA15* overexpression lines (*35S:HDA15-TAP15* and *35S:HDA15-TAP19*) (23) using the PHYB-dependent germination protocol (16). Seeds

were illuminated by either far-red light (PHYB inactivation, indicated as FR or dark conditions) or far-red light followed with red light (PHYB activation, referred as FR/R or light conditions), and then kept in the dark for 5 days (Figure 1A). As a control, seeds were kept under continuous white light (WL) and scored for germination. Upon PHYB-activated conditions, the wild-type (Col-0) and *hda15-1* seeds germinated well, while both *35S:HDA15-TAP15* and *35S:HDA15-TAP19* seeds showed significantly lower germination rates (Figure 1B and C). We next analyzed the germination of *hda15-1* under PHYB-inactivated conditions. After different intensity of FR exposure, *hda15-1* seeds showed relative higher germination rates compared with wild-type, whereas *HDA15* overexpression lines displayed relatively lower germination rates compared with wild-type (Figure 1D). Collectively, our data revealed that *HDA15* is a negative component in PHYB-mediated seed germination.

Inhibition of light-initiated seed germination by *HDA15* is dependent on PIF1

To investigate the genetic relationship between *HDA15* and *PHYB* in seed germination, we generated *hda15 phyB* double mutants by genetic crossing *hda15-1* and *phyB-9* alleles (35). As reported previously (6), the *phyB* seeds could not germinate under both FR and FR/R conditions, whereas inactivation of *HDA15* in *phyB* background partially recovered the phenotype of *phyB* mutant (Figure 2A and B), suggesting that *HDA15* might act downstream of *PHYB* in light-initiated seed germination.

Previous studies have showed that PIF1 is a major negative component in PHYB-dependent seed germination, as *pif1* mutants constitutively germinate in PHYB inactivation conditions (13). To study the genetic interaction of *HDA15* and *PIF1* in seed germination, we constructed *hda15 pif1* double mutants by crossing *hda15-1* and *pif1* alleles and generated *HDA15-GFP pif1* line by crossing *HDA15-GFP* (*35S:HDA15-GFP*) into the *pif1* knockout mutant (13,23). *hda15 pif1* and *HDA15-GFP pif1* showed similar germination rates with *pif1* seeds (Figure 2C), which were significantly higher than those of Col-0 and *HDA15-GFP* seeds under both FR and FR/R conditions (Figure 2C and D). These data suggested that inhibition of PHYB-mediated seed germination by *HDA15* is dependent on PIF1.

HDA15 directly interacts with PIF1 both *invitro* and *invivo*

The genetic interaction of *HDA15* with PIF1 in PHYB-mediated seed germination prompted us to detect whether *HDA15* could directly interact with PIF1 at the protein level. We first examined the interaction of *HDA15* and PIF1 by using yeast two-hybrid assays. As BD-PIF1 (full-length of PIF1 fused to pGBKT7) showed strong auto-activation activity, we determined the interaction of *HDA15* with the N-terminus (amino acid 1–280) and C-terminus (amino acid 281–478) of PIF1 (Figure 3A). The yeast cells co-transformed with AD-*HDA15* (full-length of *HDA15* fused to pGAKT7) and BD-PIF1 (amino acid 1–280) could grow on the selective medium QDO (synthetic medium lacking tryptophan, leucine, histidine and adenine), indicating a direct interaction between *HDA15* and PIF1 (Figure 3B). Furthermore, the N-terminus region of *HDA15*

(amino acid 1–146) is responsible for the interaction with PIF1 (Figure 3B).

Next, we determined the interaction of *HDA15* and PIF1 by using *in vitro* pull-down assays. Purified PIF1-His protein was pulled down by *HDA15*-GST (glutathione S-transferase) (Figure 3C), confirming that *HDA15* physically interacts with PIF1 *in vitro*.

The interaction of *HDA15* and PIF1 was further examined *in vivo* by bimolecular fluorescence complementation (BiFC) and co-immunoprecipitation (Co-IP) assays. For the BiFC assays, *HDA15* and PIF1 were fused to the YN vector (pEarley gate-YN) or the YC vector (pEarley gate-YC). The YN and YC constructs were co-delivered into tobacco cells by *Agrobacterium*-mediated transformation. Strong yellow fluorescence protein (YFP) signals were observed in the tobacco cells (Figure 3D), suggesting that *HDA15* can interact with PIF1 in planta.

For the Co-IP assays, the seeds of Col-0 and *TAP-PIF1* (*35S:TAP-PIF1*, expressing PIF1 protein fused with myc and his tags) (36) were treated with FR or FR/R irradiation and kept in darkness for 12 h for protein extraction. An anti-*HDA15* anti-body (23) was used for immunoprecipitation, and an anti-myc antibody was then used for western blotting analysis. As shown in Figure 3E, PIF1 protein was only detected in FR, but not FR/R conditions in imbibed seeds. Furthermore, we showed that the PIF1 protein was immunoprecipitated by the anti-*HDA15* anti-body under FR conditions, suggesting that *HDA15* and PIF1 may specifically interact in FR conditions and the interaction is diminished by the degradation of PIF1. Collectively, our results indicated that *HDA15* interacts with PIF1 both *in vitro* and *in vivo*.

Genome-wide transcriptome analysis of *HDA15*-regulated genes in imbibed seeds in dark (FR) conditions

To understand the function of *HDA15* and PIF1 in PHYB-dependent transcription regulation during seed germination, we analyzed the *HDA15*-regulated transcriptome changes by mRNA deep sequencing. Previous analysis of PIF1-regulated transcriptome changes showed that PIF1 mainly regulates gene expression in dark (FR) conditions, since PIF1 accumulates in FR conditions in imbibed seeds (12,18). We analyzed the transcriptome changes in *hda15-1* under FR conditions. After 5 min FR irradiation, seeds of Col-0 and *hda15-1* incubated in the dark for 12 h were harvested (Supplementary Figure S1A). Three biological samples were subjected to library construction and high-throughput sequencing. We performed a cutoff by 1.5-fold change with statistically significant *P*-value < 0.05. As a result, 518 genes were upregulated whereas 251 genes were downregulated in the *hda15* mutant compared with Col-0 (Supplementary Dataset 1 and 2). HDACs are identified to be the components of multi-protein co-repressor complexes and the HDAC co-repressor complexes act to remove the acetyl group from the histone tail of the target genes, leading to chromatin condensation and transcription repression (37). Therefore, *HDA15* may directly repress the expression of some of the upregulated genes by histone deacetylation, but indirectly regulate the downregulated genes in imbibed seeds. To validate the RNA-seq data, we also performed

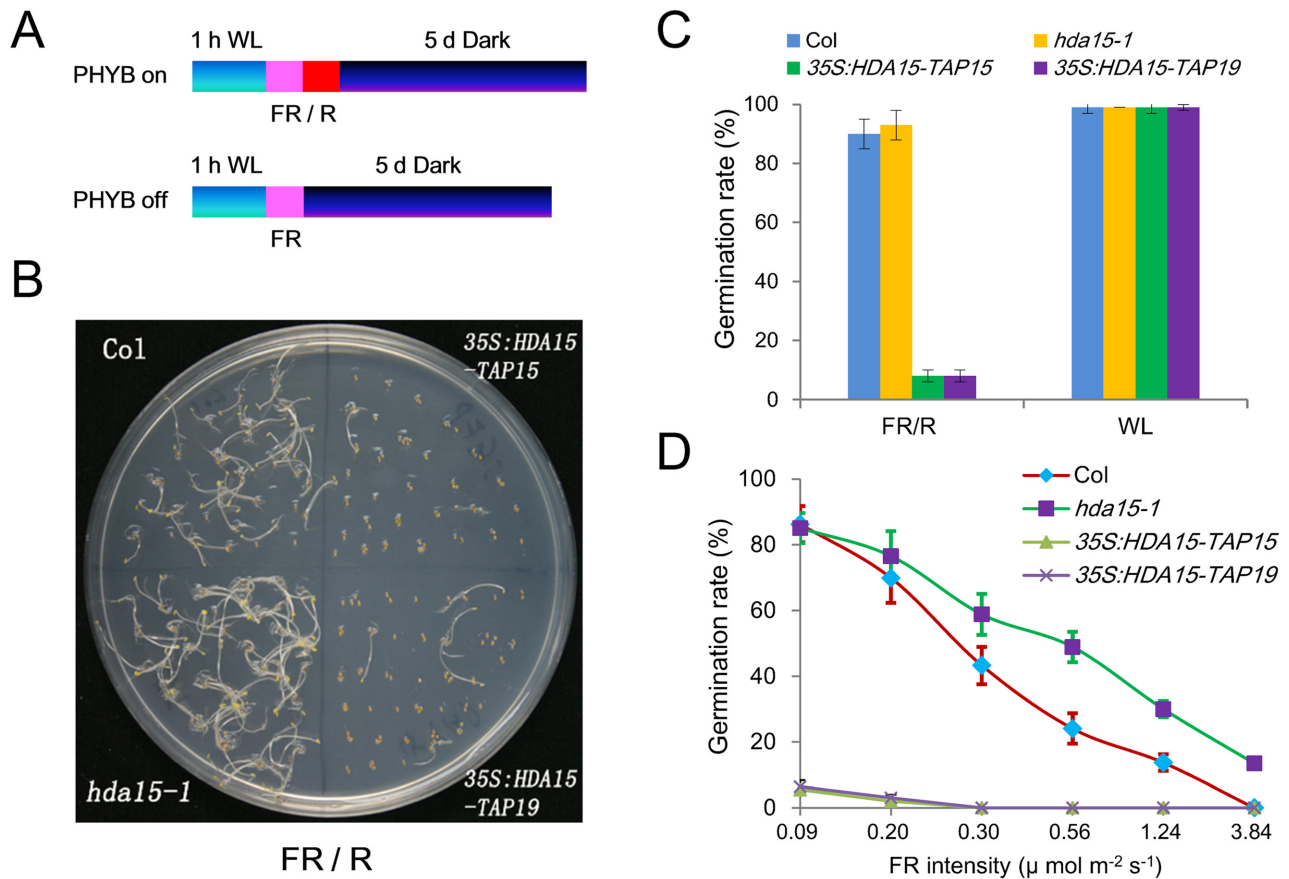


Figure 1. HDA15 is a negative regulator of PHYB-mediated promotion of seed germination. (A) Germination protocols of PHYB inactivated (PHB-off, FR) and activated (PHYB-on, FR/R) assays. FR, 5 min of far-red light ($3.82 \mu\text{mol m}^{-2} \text{s}^{-1}$), FR/R, 5 min of far-red light ($3.82 \mu\text{mol m}^{-2} \text{s}^{-1}$) and then 5 min of red light ($13.12 \mu\text{mol m}^{-2} \text{s}^{-1}$). The imbibed seeds were kept under WL for 1 h and were subsequently irradiated with FR or FR/R and kept in dark for 5 days. (B) Germination patterns of Col-0, *hda15-1* and *HDA15* overexpression lines, *35S:HDA15-TAP15* and *35S:HDA15-TAP19* on PHYB-activated conditions (FR/R). (C) Germination rate of Col-0, *hda15-1*, *35S:HDA15-TAP15* and *35S:HDA15-TAP19* on PHYB-activated conditions (FR/R). (D) Germination rates of Col-0, *hda15-1*, *35S:HDA15-TAP15* and *35S:HDA15-TAP19* on various PHYB-inactivated conditions (FR). FR light intensities were as indicated.

quantitative RT-PCR (qRT-PCR) analyses and validated a subset of randomly selected genes (Supplementary Figure S1B).

To reveal the major functional categories regulated by HDA15 in imbibed seeds, we performed GO enrichment analysis using BINGO (32). We found that the genes down-regulated in *hda15* are mainly enriched in catalytic and motor activity (Supplementary Figure S1C). In contrast, the up-regulated genes in *hda15* are significantly enriched in biotic and abiotic stress responses, as well as response to light stimuli (Supplementary Figure S1D). Elevated expression of the environmental stimulus genes in *hda15* supports the role of HDA15 in modulating endogenous responses, which allows the emerging seedlings to adapt to external environmental changes during seed germination. The proteins mediating cell wall loosening and modification function in endosperm-mediated processes during early seed germination, contributing to testa rupture (38). Remarkably, we showed that HDA15-repressed genes are also associated with plant cell wall loosening and modification (Supplementary Figure S1D), supporting the role of HDA15 in regulating testa rupture during seed germination. More-

over, HDA15 also represses the expression of the genes involved in response to auxin stimuli (Supplementary Figure S1D), indicating that HDA15 may repress seed germination through the auxin signaling pathway. Collectively, the GO analysis revealed that HDA15 may play roles in regulating the endogenous responses to adapt to external environmental changes, modulating plant cell wall loosening and modification and regulating auxin signaling transduction during seed germination.

HDA15 represses GA biosynthesis genes in imbibed seeds in the FR conditions

RNA-seq analysis demonstrated that the expression of *GA3OX1* and *GA3OX2*, two GA anabolic genes, were up-regulated in *hda15* compared with wild-type in FR conditions (Supplementary Dataset 1). We further detected the expression of these genes in imbibed wild-type and *hda15* seeds under both FR and FR/R conditions by qRT-PCR assays. Relatively higher expression levels of *GA3OX1* and *GA3OX2* were detected in FR/R compared in FR conditions in wild-type (Figure 4A). Furthermore, in contrast in FR conditions, the expression of *GA3OX1* and *GA3OX2*

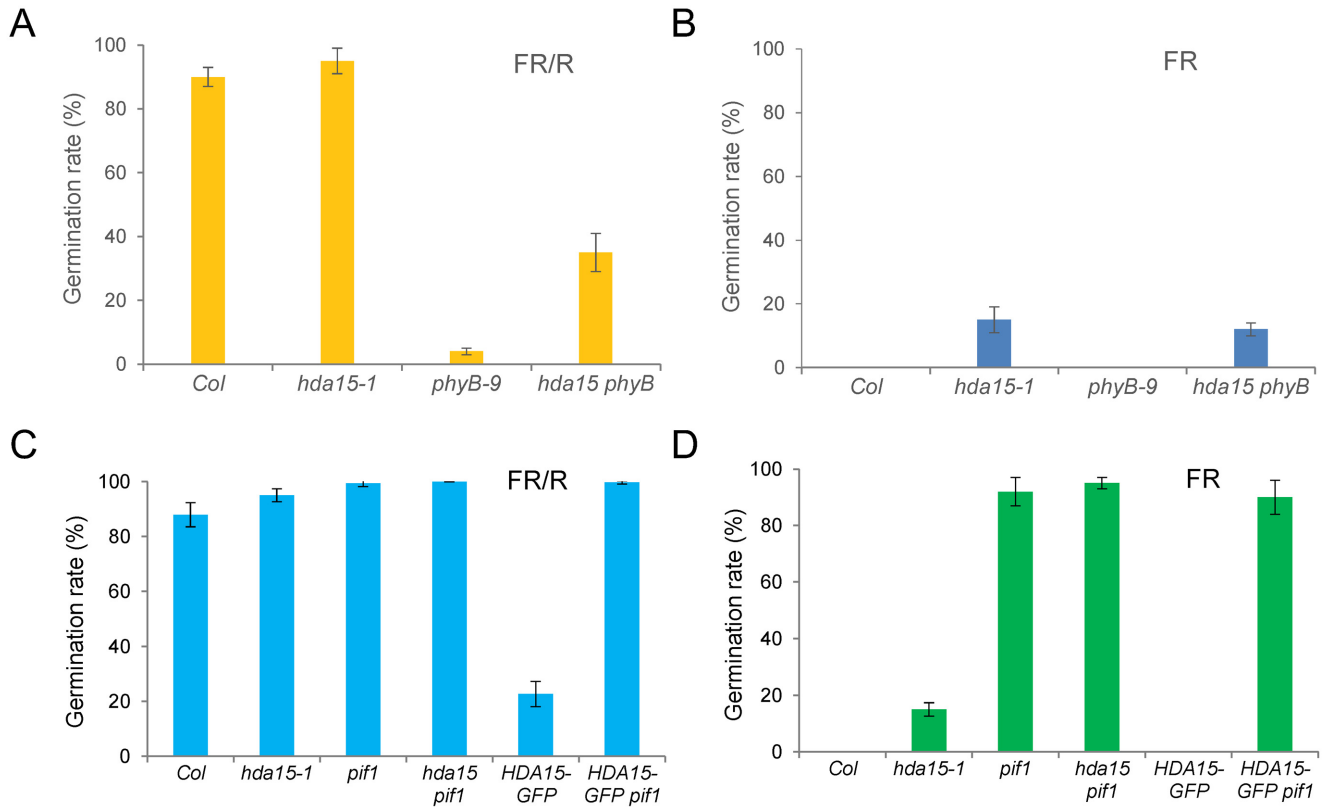


Figure 2. HDA15 acts genetically downstream of PHYB and the repression of seed germination by HDA15 is dependent on PIF1. (A) Germination frequencies of Col-0, *hda15-1*, *phyB-9* and *hda15 phyB* double mutant seeds on PHYB-activated conditions (FR/R). (B) Germination frequencies of Col-0, *hda15-1*, *phyB-9* and *hda15 phyB* double mutant seeds on PHYB-inactivated conditions (FR). (C) Germination rates of Col-0, *hda15-1*, *pif1*, *hda15 pif1*, *HDA15-GFP* (35S:*HDA15-GFP*) and *HDA15-GFP pif1* on PHYB-activated conditions (FR/R). (D) Germination rates of Col-0, *hda15-1*, *pif1*, *hda15 pif1*, *HDA15-GFP* (35S:*HDA15-GFP*) and *HDA15-GFP pif1* on PHYB-inactivated conditions (FR).

was not upregulated in *hda15* compared with wild-type in FR/R conditions (Figure 4A), which indicating that HDA15 may repress GA biosynthesis in FR conditions only.

Next, we analyzed the histone acetylation levels of *GA3OX1* and *GA3OX2* in FR/R and FR conditions in wild-type by ChIP assays. Relatively higher levels of histone H3 acetylation in the promoter and first exon regions of *GA3OX1* and *GA3OX2* were detected in FR/R compared to in FR conditions, indicating that the activation of *GA3OX1* and *GA3OX2* in FR/R conditions is correlated with histone acetylation (Figure 4B). To examine whether HDA15 targets to these genes *in vivo*, the ChIP assays were performed with imbibed Col-0 and *HDA15-GFP* (35S:*HDA15-GFP*) seeds under FR conditions. The enrichment of fragments of the promoter regions of *GA3OX1* and *GA3OX2* was higher in *HDA15-GFP* seeds compared with Col-0 (Figure 4C). Collectively, these data suggested that HDA15 directly represses GA biosynthesis genes *GA3OX1* and *GA3OX2* in imbibed seed in FR conditions.

HDA15 and PIF1 co-repress plant hormones and cellular function-related genes in FR conditions

Previous work identified that 1112 and 44 genes are regulated by PIF1 under FR (dark) and FR/R conditions,

respectively (18). We then compared HDA15 and PIF1 co-regulated genes under FR conditions. The seeds were treated in the same condition as reported (18). As a result, we showed that 267 genes are co-regulated by HDA15 and PIF1 in imbibed seeds under FR conditions (Supplementary Dataset 3). Among which, 246 (92.1%) genes are up-regulated and 12 (4.5%) genes are downregulated in both *hda15* and *pif1* mutants (Figure 5A and Supplementary Dataset 3). Only 9 (3.4%) genes are differentially regulated in *hda15* and *pif1* mutants. Together, these data suggested that HDA15-PIF1 may act together as transcriptional regulators in imbibed seeds.

We then compared the HDA15 and PIF1 co-regulated genes with those genes regulated by light (Col-0 FR/R/Col-0 FR) (18). Very similar transcriptome changes were identified when comparing *HDA15*, *PIF1* and light-regulated genes (Figure 5B). Furthermore, most (95.3%) of HDA15-PIF1 repressed genes are also light-induced genes (Figure 5C and Supplementary Dataset 4), suggesting that the HDA15-PIF1 module plays an important role in the transcriptional repression of the light responsive genes in imbibed seeds. GO analysis by using the DAVID (for Database for Annotation, Visualization and Integrated Discovery) resource (33) revealed that HDA15 and PIF1 co-repressed genes are associated with cell growth, regulation

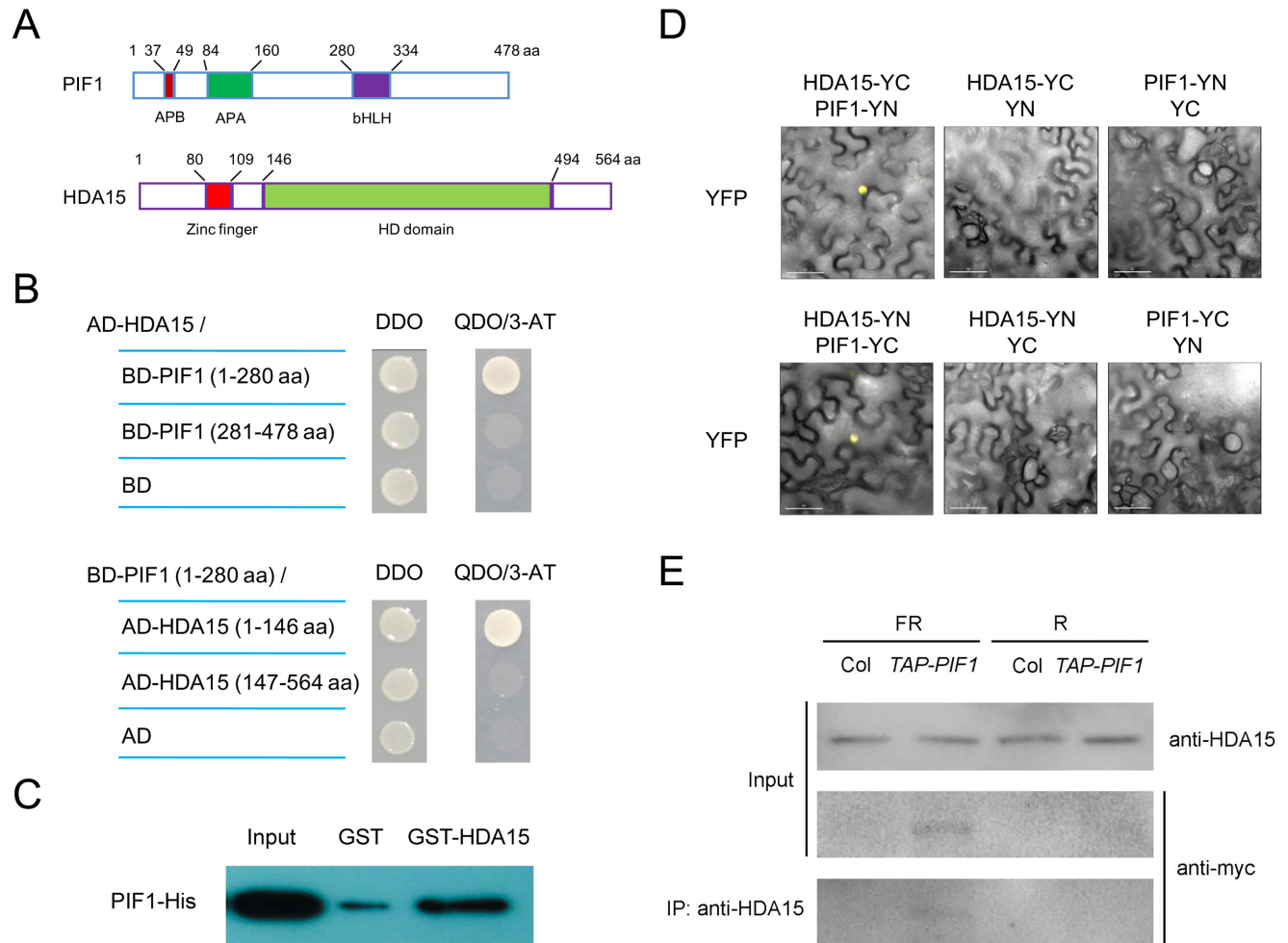


Figure 3. HDA15 interacts with PIF1 both *in vitro* and *in vivo*. (A) Schematic structures of PIF1 and HDA15 protein domains. aa, amino acids. (B) Yeast two-hybrid analysis of HDA15–PIF1 interaction. The different regions of HDA15 and PIF1 fused with AD and BD vectors were co-transformed into yeast cells and plated on DDO. The transformants were then plated on QDO supplemented with 20 mM 3-AT (3-amino-1,2,4-triazole) to test for possible interaction. DDO, SD/-Leu/-Trp, QDO, SD/-Leu/-Trp/-His/-Ade. (C) *In vitro* pull-down analysis of HDA15–PIF1 interaction. PIF1-His protein was incubated with HDA15-GST or GST, the bounded proteins were then detected by western blotting assays using an anti-His antibody. (D) BiFC analysis of the interaction of HDA15 with PIF1 in tobacco leaf cells. HDA15 and PIF1 were fused with the C-terminus (YC) or the N-terminus (YN) of YFP and co-transformed into tobacco epidermal cells by PEG-mediated transformation. Empty vectors were used as negative controls. (E) Co-IP analysis of the interaction of HDA15 with PIF1 in imbibed seeds. Seeds of Col-0 and *TAP1-PIF1* were treated with FR and FR/R and kept in the dark for 12 h. The extracted protein was immunoprecipitated with an anti-HDA15 antibody and then detected by western blotting assays with an anti-myc antibody.

of cell sizes, cell walls, hydrolases and response to hormone and abiotic stress stimuli (Figure 5D).

A large subset of auxin signaling components, including auxin efflux carriers *PINs*, auxin influx transporter *AUX1*, auxin responsive transcription factors *IAAs* and SAUR-like auxin-responsive proteins *SAURs* were co-repressed by HDA15 and PIF1 (Figure 6A and Supplementary Dataset 3). To validate the data, we detected the expression levels of *PIN1*, 2, 7 and *AUX1* in *hda15*, *pif1* and *hda15 pif1* double mutant seeds under FR conditions. qRT-PCR analysis confirmed that these genes were co-repressed by HDA15 and PIF1 (Figure 6B). Furthermore, we showed that HDA15-PIF1 co-regulated the expression of genes involved in GA and ABA signaling pathways. The expression levels of GA biosynthesis genes, *GA3OX1* and *GA3OX2*, were upregulated in both *hda15* and *pif1* mutants, which is consis-

tent with their elevated germination rates under PHYB-inactivated conditions. Interestingly, we also showed that HDA15 and PIF1 co-repressed the expression levels of ABA receptors *PYLs*, ABA-responsive transcription factor *ABI4* and ABA catabolism gene *CYP707A2* (Figure 6A), suggesting that HDA15-PIF1 may co-regulate gene expression in ABA biosynthesis and signaling pathways. In addition, several genes involved in brassinolide, cytokinin and ethylene signal pathways were also co-regulated by HDA15 and PIF1 (Figure 6A).

Loosening of cell walls is a crucial development process during seed germination. Xyloglucan Endotransglucosylases/Hydrolases (XTHs), Expansins (EXPs) and Pectinesterases/Pectin Methylsterases (PMEs) are key enzymes involved in cell wall loosening and cell expansion (1,39). A number of *XTHs*, *EXPs* and

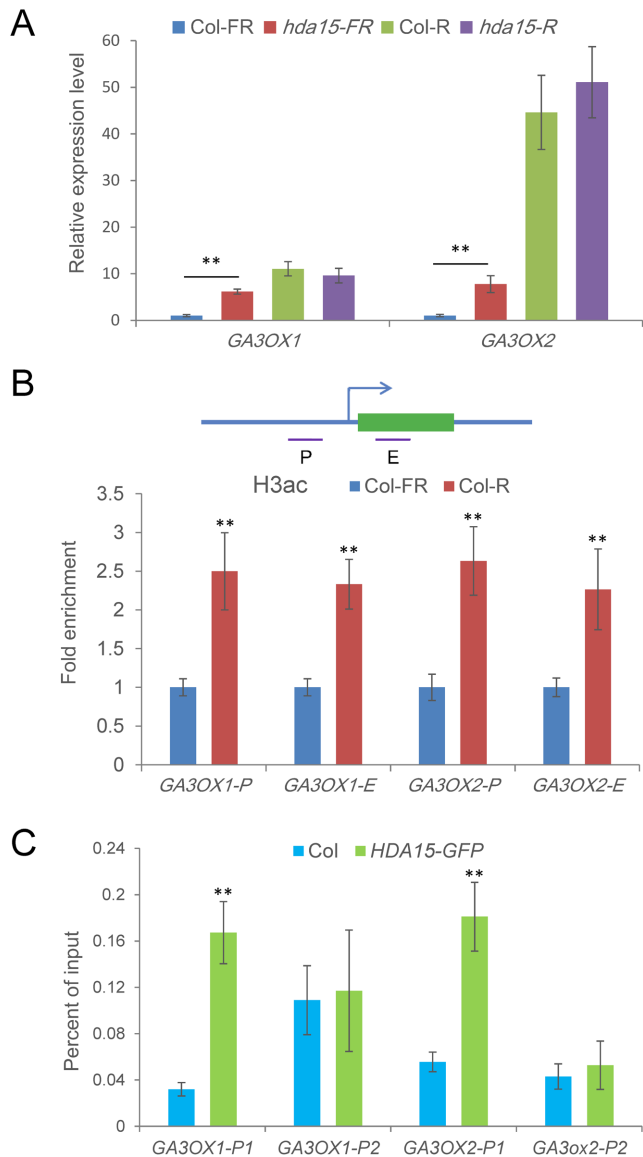


Figure 4. HDA15 directly represses *GA3OX1* and *GA3OX2* expression by histone deacetylation. (A) qRT-PCR analysis of the expression levels of *GA3OX1* and *GA3OX2* in Col-0 and *hda15* seeds under FR and FR/R conditions. *PP2A* was used as an internal control. Values are shown as means \pm SD (*t*-test, **P* < 0.05, ***P* < 0.01). (B) ChIP-qPCR analysis of the histone H3 acetylation levels in the promoters (P) and exons (E) of *GA3OX1* and *GA3OX2* under FR and R conditions. *ACTIN2* was used as an internal control, the relative histone H3 acetylation levels of these genes were normalized to *ACTIN2*. Values are shown as means \pm SD (*t*-test, **P* < 0.05, ***P* < 0.01). (C) ChIP-qPCR analysis of enrichment of HDA15 in the promoter regions of *GA3OX1* and *GA3OX2* under FR conditions. The Col-0 and *HDA15-GFP* seeds were used for analysis. An anti-GFP antibody was used for immunoprecipitation. P1 and P2 indicate different regions in the promoters of *GA3OX1* and *GA3OX2*. Values are shown as means \pm SD (*t*-test, **P* < 0.05, ***P* < 0.01).

PMEs were shown to be co-repressed by HDA15 and PIF1 (Figure 6A). qRT-PCR analysis confirmed that the expression of *XTH4*, 5, 9, 16 and *EXP1*, 3, 9, 15 were upregulated in *hda15*, *pif1* and *hda15 pif1* double mutants (Figure 6B), suggesting that HDA15 and PIF1 may repress gene expression involved in cell wall loosening and cell

expansion. Furthermore, we showed that HDA15 and PIF1 co-represses the genes involved in cell cycle, cellular transport and cell differentiation (Figure 6A). Collectively, these findings revealed that HDA15-PIF1 co-regulate gene expression involved in multiple cellular processes and pathways.

PIF1 and HDA15 co-target to the promoters of auxin transport and cell wall loosening-related genes in FR conditions

The RNA-seq data showed that HDA15-PIF1 co-represses a large number of genes including the auxin signaling and cell wall loosening-related genes. We further tested whether these genes are direct targets of HDA15 and PIF1 in imbibed seed. Since PIFs preferentially binds to the G-box (CACGTG) and PBE-box (CACATG) motifs in the target promoters (40), we selected those genes such as *PINs*, *XTHs* and *EXPs* containing at least one G-box motif in their promoters for further analysis. Transgenic plants expressing *PIF1-GFP* were generated and used for ChIP assays. Expression of *PIF1-GFP* in *pif1* background fully recovered the hypocotyl length and germination phenotypes of *pif1* under red light and PHYB inactivation conditions (Figure 7A), suggesting that *PIF1-GFP* is functional *in vivo*. Consistent with the previous report (12), we showed that PIF1 protein was accumulated in imbibed seeds under FR conditions, but was degraded under FR/R conditions (Supplementary Figure S2). As reported previously, ChIP analysis revealed that PIF1 bound to the G-box containing promoter region of *RGA* under FR conditions (16). Furthermore, PIF1 also bound to the G-box containing promoter regions of *EXP1*, *EXP3*, *EXP9*, *XTH4*, *XTH16*, *PIN1* and *PIN3* under FR conditions. However, the enrichment of PIF1 to these target genes was diminished under FR/R conditions (Figure 7B). These data revealed that red light exposure induces PIF1 degradation thus decreases the enrichment of PIF1 to the target genes in imbibed seeds.

To examine whether HDA15 also associates with these genes *in vivo*, the ChIP assays were performed with imbibed Col-0 and *HDA15-GFP* (35S:*HDA15-GFP*) seeds under FR conditions. The enrichment of the G-box containing promoter regions of *EXP1*, *EXP3*, *EXP9*, *XTH4*, *XTH16*, *PIN1* and *PIN3* was higher in *HDA15-GFP* seeds compared with Col-0 (Figure 7C). Furthermore, we showed that the binding of HDA15 was decreased in *HDA15-GFP pif1* compared with *HDA15-GFP* seeds under FR conditions (Figure 7C), indicating that the association of HDA15 with these targets is dependent on PIF1. Collectively, these data revealed that HDA15 and PIF1 co-target to the auxin-responsive and cell wall loosening-related genes under FR conditions in imbibed seeds.

HDA15 and PIF1 decrease the histone H3 acetylation levels of the target genes in FR conditions

Previous study reported that HDA15 has HDAC activity (23). To determine whether HDA15-PIF1 represses their target genes by histone deacetylation, we compared the expression and histone acetylation levels of the HDA15-PIF1 target genes under the FR and FR/R conditions. Relatively high expression levels of *EXPs*, *XTHs* and *PINs* were de-

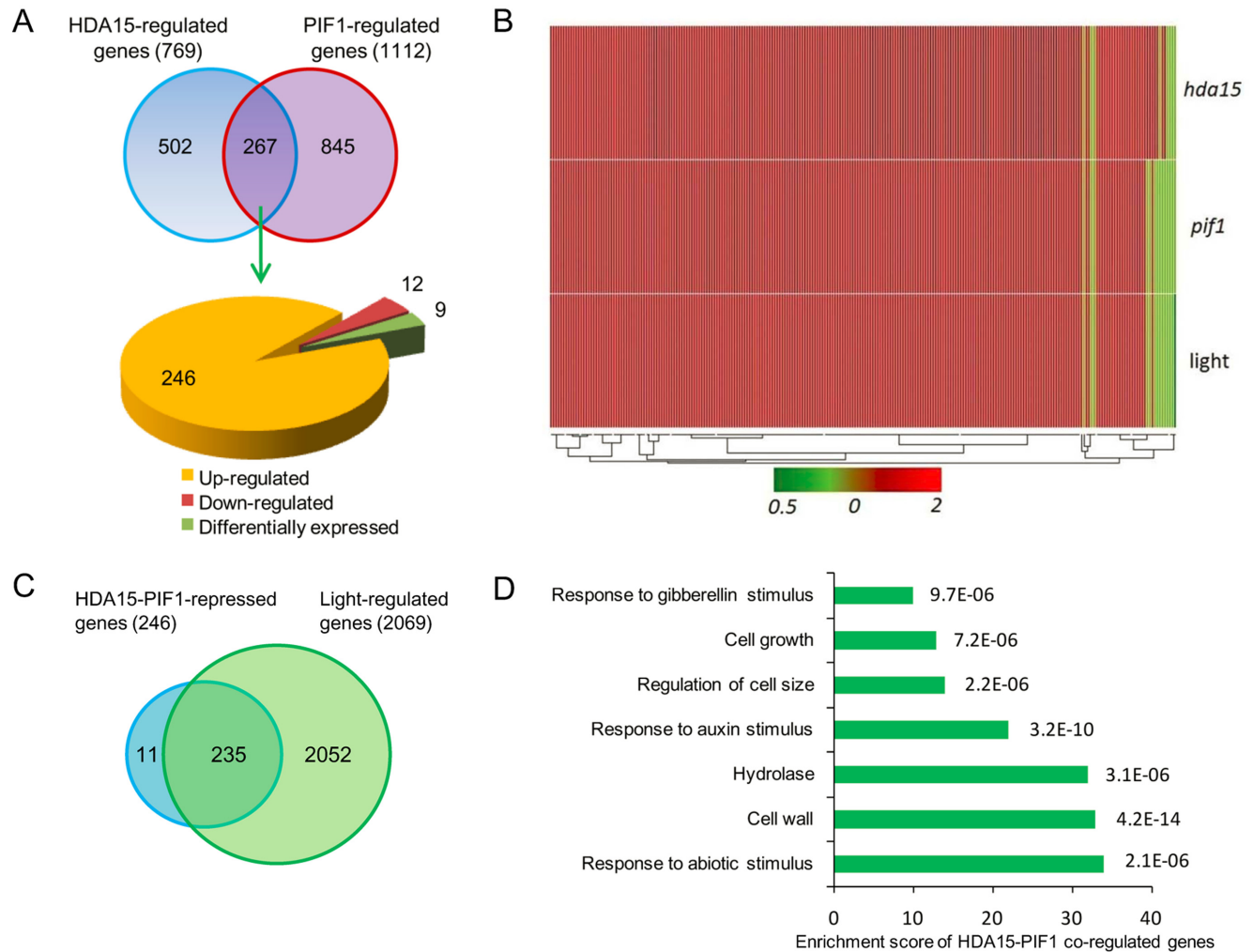


Figure 5. HDA15 and PIF1 co-regulate the transcriptional network in light-initiated seed germination process. (A) Venn diagram shows the overlap of HDA15 and PIF1 co-regulated genes. (B) Cluster analysis of HDA15-, PIF1- and light-regulated genes. The bar indicates the fold change. (C) Venn diagram displays the overlap of HDA15-PIF1-repressed and light-regulated genes. (D) DAVID functional clustering of HDA15-PIF1 co-regulated genes. The *P*-values corresponding to the categories are as indicated.

tected in FR/R conditions, which was associated with increased H3 acetylation levels in the promoters (P) and the first exons (E) of these genes (Figure 8A and B), indicating that these genes are regulated by light-mediated histone acetylation changes.

Next, we analyzed the histone acetylation levels of the HDA15-PIF1 target genes in Col-0, *hda15*, *pif1* and *hda15 pif1* double mutants under FR conditions. The H3 acetylation levels in the promoter and first exon regions of these genes were increased in *hda15*, *pif1* and *hda15 pif1* seeds compared with wild-type (Figure 8C), supporting that HDA15-PIF1 represses their target gene expression by decreasing the histone acetylation levels.

DISCUSSION

Light is one of the most important environmental factors affecting the initiation of seed germination. Here, we present evidence indicating that HDA15 is a negative reg-

ulator in light-dependent seed germination. HDA15 acts downstream of PHYB and the repression of seed germination by HDA15 is dependent on PIF1. Moreover, we found that HDA15 specifically interacts with PIF1 in FR (or dark) conditions to repress gene expression involved in multiple plant hormones and cellular function processes via decreasing the histone acetylation levels. Our findings revealed that HDA15-PIF1 acts as a key repression module directing the transcriptional network in light-initiated seed germination process.

HDA15 and PIF1 integrate multiple hormonal signaling in repression of light-initiated seed germination

Previous studies revealed that PIF proteins function as a pivotal component in a cellular signaling hub that integrates multiple signals to orchestrate the transcriptional network that drives multiple facets of development processes (41). Beside light, seed germination is also regulated by various

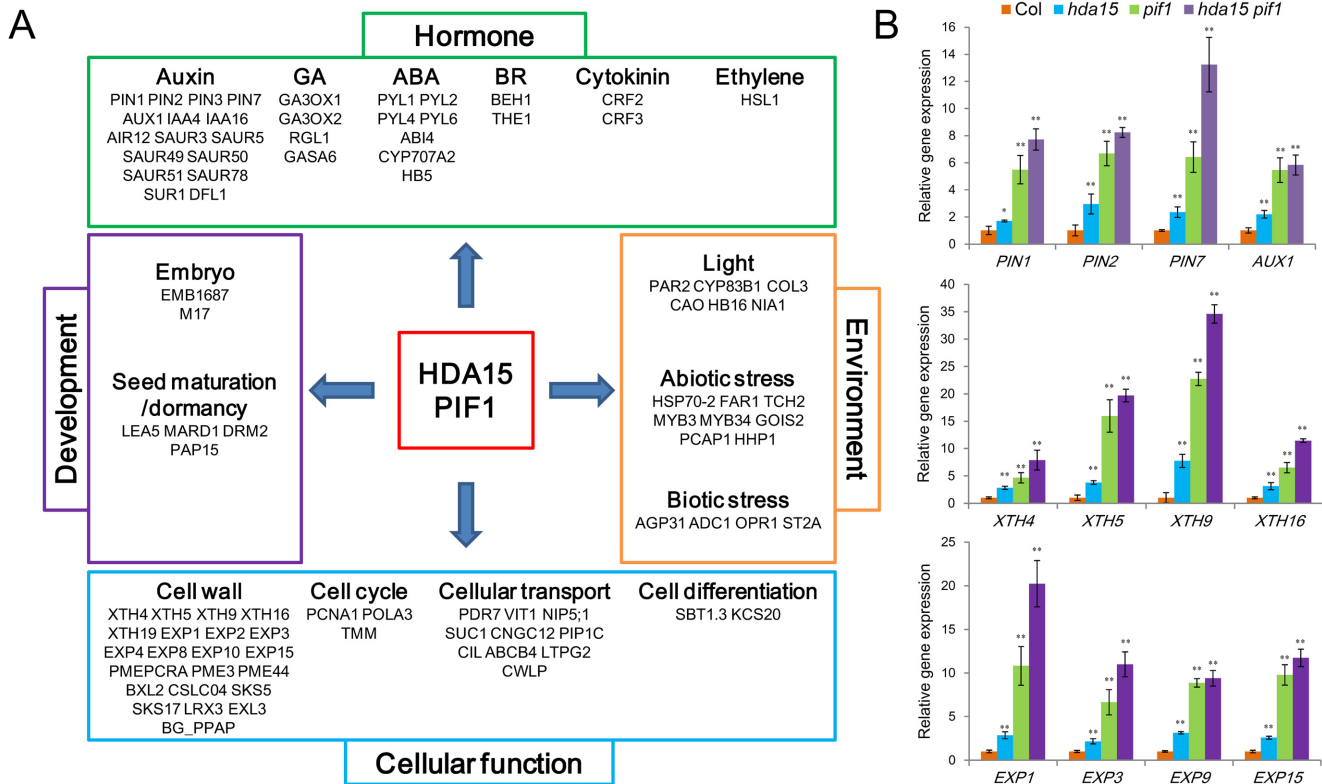


Figure 6. HDA15-PIF1 co-represses the genes involved in plant hormones and cellular processes in imbibed seeds. (A) HDA15-PIF1 co-repressed genes related to plant hormones and cellular processes. (B) qRT-PCR analysis of the expression levels of *PINs*, *XTHs* and *EXPs* in Col-0, *hda15*, *pif1* and *hda15 pif1* seeds in FR conditions. *PP2A* was used as an internal control. Values are shown as means \pm SD (*t*-test, **P* < 0.05, ***P* < 0.01, difference from Col-0).

endogenous factors such as plant hormones (42–50). GA and ABA are two most important plant hormones which act antagonistically on seed germination. ABA is involved in establishing and maintaining seed dormancy, whereas GA is involved in breaking seed dormancy and promoting germination (1). In present work, RNA-seq analysis demonstrated that HDA15 and PIF1 co-repress the expression of two GA anabolic genes, *GA3OX1* and *GA3OX2*. We further showed a direct association of HDA15 with the promoters of *GA3OX1* and *GA3OX2* in FR conditions. Furthermore, activation of *GA3OX1* and *GA3OX2* by FR/R treatment is correlated with the increase of histone H3 acetylation levels. These findings suggested that HDA15 may repress light-initiated seed germination at least partially by direct regulating of the GA biosynthesis pathway. Since *GA3OX1* and *GA3OX2* are not directly bound by PIF1 (16), HDA15 may repress their expression independent of PIF1.

HDA15 and PIF1 may also play a role in the ABA signaling pathway since they co-repress the expression of the ABA catabolic gene *CYP707A2* in imbibed seeds. Previous work displayed that *CYP707A2* is responsible for reducing ABA levels from late-maturation to germination (51). HDA15 and PIF1 may repress light-initiated seed germination by increasing ABA levels. In addition, we showed that HDA15 and PIF1 also co-repress the expression of a subset of ABA receptor genes, *PYL1*, *PYL2*, *PYL4* and *PYL6* (52,53). Although the role of these *PYLs* in seed germina-

tion is not characterized, our data confirmed an important role of HDA15-PIF1 in ABA signaling pathway.

Auxin is a key plant hormone regulating cell cycling, plant growth and development (54). However, the exact role of auxin in seed germination is unclear. In present work, we showed that HDA15 and PIF1 co-repress a number of genes in the auxin signaling pathway, including auxin efflux carriers *PINs*, the auxin influx transporter *AUX1*, auxin responsive transcription factors *IAAs* and SAUR-like auxin-responsive proteins *SAURs*, suggesting that these genes may play important roles in light-initiated seed germination. Furthermore, more recent studies revealed a direct link between auxin and light signaling by which PIF4 interacting with the auxin responsive transcription factor ARF8 (55). In addition, PIF4 and ARF8 also share common target genes, such as *AUX/IAAs*, *SAURs* and *PINs* (55). We found that some members of *PINs* and *AUX/IAAs* are also the direct targets of HDA15 and PIF1. Therefore, HDA15, PIF1 and ARFs may form a protein complex that integrates light and auxin signal pathways in regulating seed germination. Further work is needed to elucidate the crosstalk of HDA15, PIFs and ARFs in light-dependent seed germination.

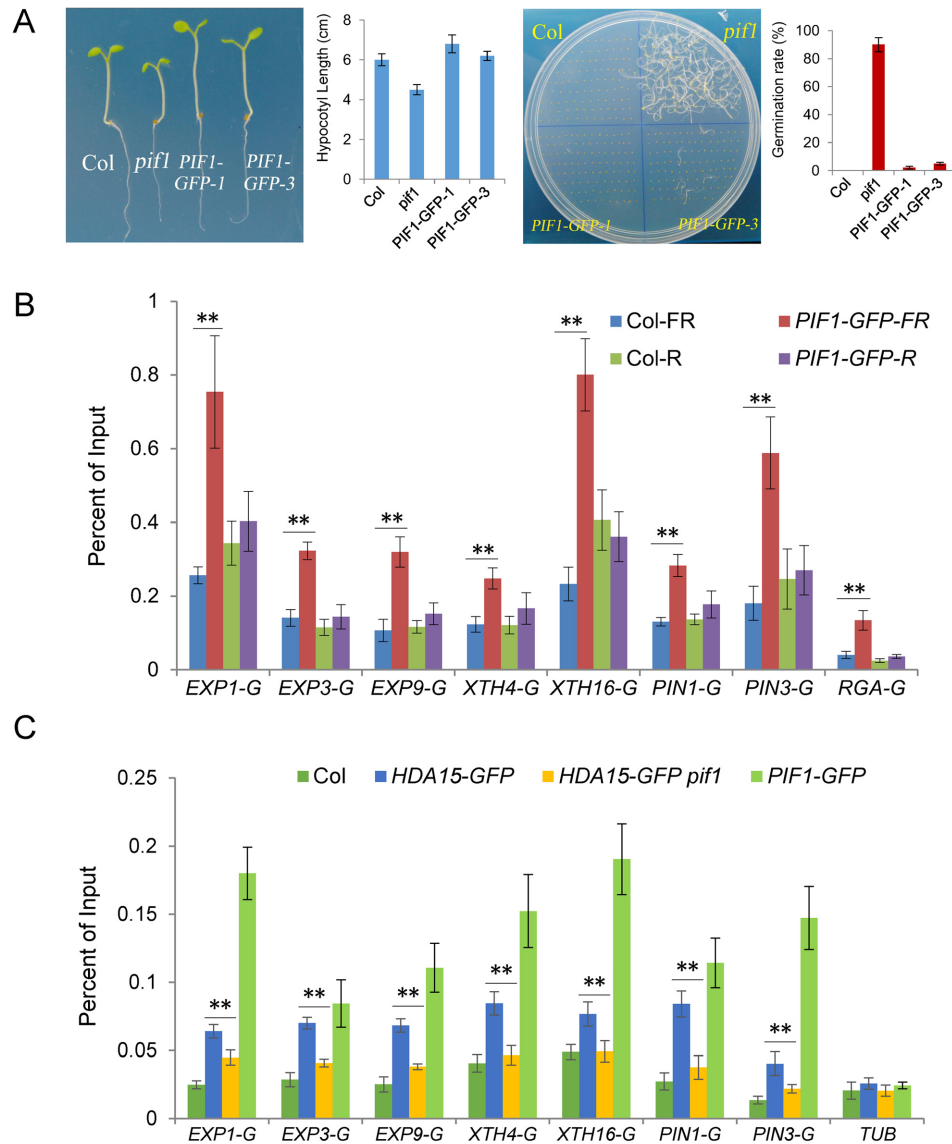


Figure 7. PIF1 and HDA15 co-target to the promoters of auxin transport and cell loosening-related genes. (A) Phenotypic analysis of PIF1-GFP transgenic lines. *PIF1-GFP* lines were constructed in *pif1* background. The hypocotyls of *PIF1-GFP-1* and *PIF1-GFP-3* were measured after grown under continuous red light conditions ($13.12 \mu\text{mol m}^{-2} \text{s}^{-1}$). The germination rates of *PIF1-GFP-1* and *PIF1-GFP-3* were scored after PHYB-off treatment (FR, $3.82 \mu\text{mol m}^{-2} \text{s}^{-1}$). ($n > 30$). (B) ChIP-qPCR analysis of enrichment of PIF1 in the G-box containing regions in the promoters of *PINs*, *XTHs* and *EXPs* under FR and FR/R conditions. The *PIF1-GFP-1* line was used for analysis. An anti-GFP antibody was used for ChIP assay. Values are shown as means \pm SD (*t*-test, $*P < 0.05$, $**P < 0.01$). (C) ChIP-qPCR analysis of enrichment of HDA15 in the G-box containing regions of the promoters of *PINs*, *XTHs* and *EXPs* in imbibed seeds of *HDA15-GFP*, *HDA15-GFP pif1* and *PIF1-GFP* under FR conditions. An anti-GFP antibody was used for immunoprecipitation. Values are shown as means \pm SD (*t*-test, $*P < 0.05$, $**P < 0.01$).

PIF1 functions as a transcriptional repressor by recruiting HDA15 in phytochrome-dependent seed germination process

Seed germination is light dependent in many plants. Previous studies have demonstrated that PHYB promotes seed germination in response to the red light LFR, whereas PHYA induces seed germination in response to the VLFR and the far-red high irradiance response (8,56). In present work, we found that loss of function of *HDA15* increases whereas gain of function of *HDA15* decreases PHYB-dependent seed germination. Furthermore, inactivation of *HDA15* in *phyB* partially recovered the phenotype of *phyB*

mutant. These findings revealed a negative role of HDA15 in the PHYB-mediated seed germination process. Moreover, we showed that *HDA15* overexpression lines displayed strong germination inhibition after the very low intensity of FR exposure, suggesting that HDA15 may also interfere with the PHYA VLFR response. Since PIF1 inhibits both PHYA- and PHYB-dependent seed germination (13,16), the HDA15-PIF1 module may, therefore, negatively regulate both PHYA- and PHYB-mediated seed germination processes.

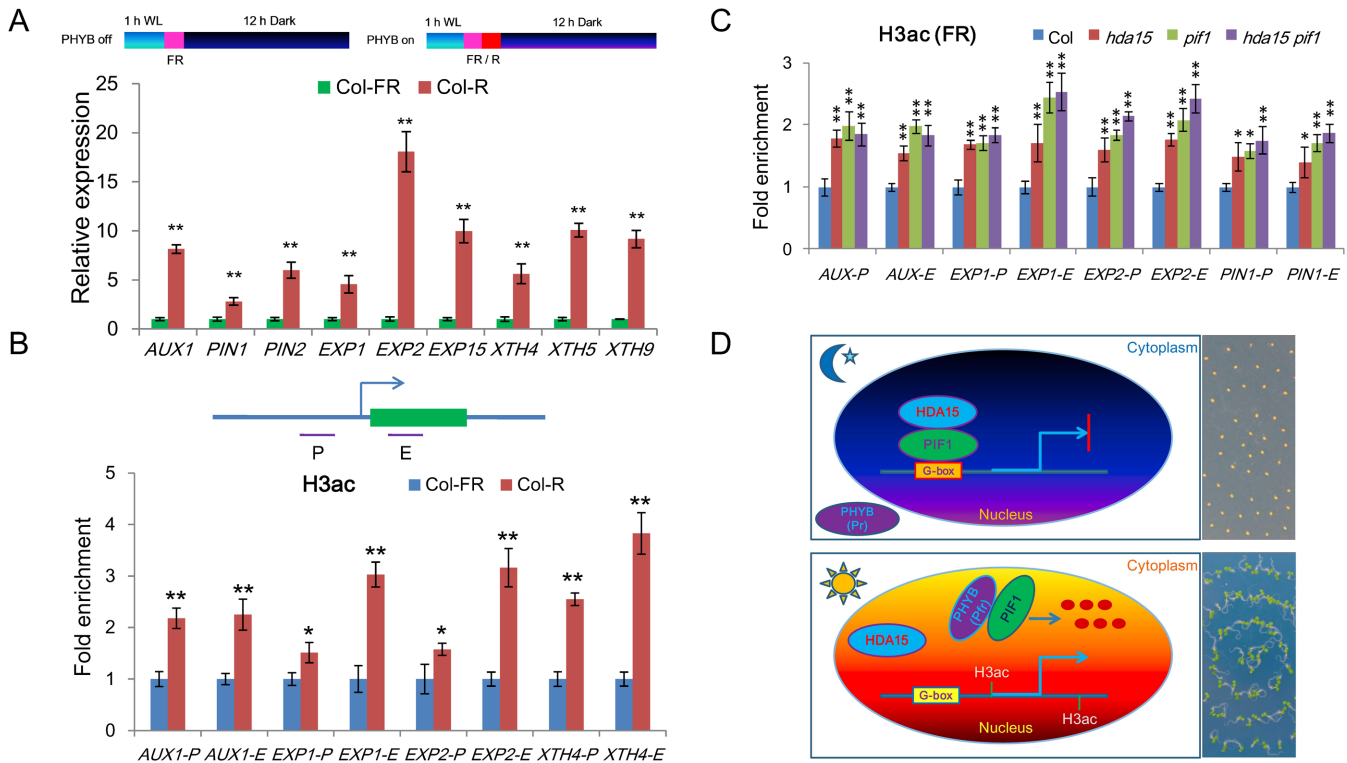


Figure 8. HDA15 and PIF1 decrease the histone H3 acetylation levels of the target genes. (A) qRT-PCR analysis of the expression levels of *PINs*, *EXPs* and *XTHs* under FR and FR/R conditions. *PP2A* was used as an internal control. Values are shown as means \pm SD (*t*-test, * P < 0.05, ** P < 0.01). (B) ChIP-qPCR analysis of the histone H3 acetylation levels in the promoters and exons of *PINs*, *EXPs* and *XTHs* under FR and R conditions. *ACTIN2* was used as an internal control, the relative histone H3 acetylation levels of these genes were normalized to *ACTIN2*. Values are shown as means \pm SD (*t*-test, * P < 0.05, ** P < 0.01). (C) ChIP-qPCR analysis of the histone H3 acetylation levels in the promoters and exons of *AUX1*, *EXP1*, *EXP2* and *PIN1* in Col-0, *hda15*, *pif1* and *hda15 pif1* under FR conditions. *ACTIN2* was used as an internal control, the relative histone H3 acetylation levels of these genes were normalized to *ACTIN2*. Values are shown as means \pm SD (*t*-test, * P < 0.05, ** P < 0.01, difference from Col-0). (D) Proposed model for the HDA15-PIF1 module in light-dependent seed germination pathway. Under FR or dark conditions, PHYB is localized in the cytosol in the Pr form, which permits PIF1 to accumulate in the nucleus. PIF1 interacts and recruits HDA15 to repress seed germination-related gene expression by decreasing the histone H3 acetylation levels, resulting in the inhibition of seed germination state. However, upon R or strong light conditions, activated form of PHYB (Pfr) translates into the nucleus and induces rapid phosphorylation and degradation of PIF1 proteins through the 26S proteasome system. The HDA15-PIF1 repression module is dissociated from the targets, resulting in the initiation of seed germination.

The PIF proteins including PIF1, PIF3, PIF4 and PIF5 act as either positive or negative regulators in distinct phytochrome-dependent development processes (57). Genome-wide binding and transcription analyses also revealed that PIFs regulate various light responses via direct activating or repressing downstream genes (17,40,58). These findings support a dual role of PIFs in transcription regulation in light response. However, it remains unclear how the transcription activity of PIFs is regulated. The functional diversity among different PIF members has also been reported. For example, PIF1 plays a dominant role in controlling seed germination, whereas PIF4 and PIF5 appear to be the central players in regulating shade avoidance (41). Previously, we found that PIF3 interacts with HDA15 to repress chlorophyll biosynthesis genes in etiolated *Arabidopsis* seedlings (23). In this study, we demonstrated that PIF1 associates with HDA15 in repression of the transcriptome network involved in multiple hormonal signaling and cellular processes in imbibed seeds. By contrast, HDA15 does not interact with PIF4 and PIF5 (23), supporting the functional diversity among different PIF members.

A direct interaction between PIF3 and PICKL, an ATP-dependent chromatin remodeling factor, was also reported (59). Moreover, PIF3 and PICKL co-activate the cell elongation-related gene expression by decreasing the H3K27me3 levels in *Arabidopsis* seedlings (59), supporting a transcription activation function of PIF3 in light response. These findings indicate that PIFs may activate or repress gene expression by interacting with transcriptional co-activators or co-repressors. Similarly, in human cells, the transcription factor Krüppel-like Factor 4 (KLF4) activates gene expression when interacting with co-activators p300 and CREB-binding proteins, but represses gene expression when interacting with co-repressors such as Histone Deacetylase3 (HDAC3) (60). Since PIF1 plays a unique role in light-initiated seed germination due to its high level of expression in imbibed seeds (61), HDA15-PIF1 might compose a specific repression module directing the transcription regulation in light-regulated seed germination process.

The PIF1 protein is stable in the dark, but it is degraded in response to light through the ubiquitin-26S proteasome pathway (14). FR/R treatment triggers a quick protein degradation of PIF1 in imbibed seeds (12). Consistent with

the PIF1 protein changes, we found that the binding of PIF1 to its target genes is high under FR conditions, but this binding is decreased upon FR/R exposure in imbibed seeds. The binding of HDA15 to the target genes is decreased in the *pif1* mutant, supporting that HDA15 may be recruited to the target loci by PIF1 in imbibed seeds. The degradation of PIF1 under FR/R conditions leads to reduced binding of HDA15 to the target loci. Therefore, R (red light) exposure induces both protein degradation of PIF1 and dissociation of HDA15 from the target genes.

In summary, we proposed a model for the function of the HDA15-PIF1 module in the light-dependent seed germination pathway. Under FR or dark conditions, PHYB is localized in the cytosol in the Pr form, which permits PIF1 to accumulate in the nucleus. PIF1 interacts and recruits HDA15 to repress the expression of seed germination-related genes by decreasing the histone H3 acetylation levels, resulting in an inhibition of seed germination. Upon R or strong light conditions, activated form of PHYB (Pfr) translocates into the nucleus and induces rapid phosphorylation and degradation of PIF1 through the 26S proteasome system. The HDA15-PIF1 repression module is then dissociated from the target genes, resulting in the initiation of seed germination (Figure 8D).

SUPPLEMENTARY DATA

Supplementary Data are available at NAR Online.

ACKNOWLEDGEMENT

We thank Technology Commons, College of Life Science, National Taiwan University for the convenient use of the Bio-Rad real-time PCR system and the confocal spectral microscope imaging system.

FUNDING

Youth Innovation Promotion Association, CAS [2014319]; Natural Science Foundation of Guangdong Province [2014A030313786]; National Natural Science Foundation of China [31301056, 31371308]; National Science Council of Taiwan [105-2311-B-002-012-MY3, 105-2313-B-002-005]. Funding for open access charge: National Natural Science Foundation of China [31301056, 31371308].

Conflict of interest statement. None declared.

REFERENCES

- Finch-Savage, W.E. and Leubner-Metzger, G. (2006) Seed dormancy and the control of germination. *New Phytol.*, **171**, 501–523.
- Weitbrecht, K., Muller, K. and Leubner-Metzger, G. (2011) First off the mark: early seed germination. *J. Exp. Bot.*, **62**, 3289–3309.
- Quail, P.H. (2002) Photosensory perception and signalling in plant cells: new paradigms? *Curr. Opin. Cell Biol.*, **14**, 180–188.
- Rizzini, L., Favory, J.J., Cloix, C., Faggionato, D., O'Hara, A., Kaiserli, E., Baumeister, R., Schäfer, E., Nagy, F., Jenkins, G.I. *et al.* (2011) Perception of UV-B by the Arabidopsis UVR8 protein. *Science*, **332**, 103–106.
- Borthwick, H.A., Hendricks, S.B., Parker, M.W., Toole, E.H. and Toole, V.K. (1952) A reversible photoreaction controlling seed germination. *Proc. Natl. Acad. Sci. U.S.A.*, **38**, 662–666.
- Shinomura, T., Nagatani, A., Chory, J. and Furuya, M. (1994) The induction of seed germination in Arabidopsis thaliana is regulated principally by phytochrome B and secondarily by phytochrome A. *Plant Physiol.*, **104**, 363–371.
- Mathews, S. (2006) Phytochrome-mediated development in land plants: red light sensing evolves to meet the challenges of changing light environments. *Mol. Ecol.*, **15**, 3483–3503.
- Botto, J.F., Sanchez, R.A., Whitelam, G.C. and Casal, J.J. (1996) Phytochrome A mediates the promotion of seed germination by very low fluences of light and canopy shade light in Arabidopsis. *Plant Physiol.*, **110**, 439–444.
- Seo, M., Nambara, E., Choi, G. and Yamaguchi, S. (2009) Interaction of light and hormone signals in germinating seeds. *Plant Mol. Biol.*, **69**, 463–472.
- Jiao, Y., Lau, O.S. and Deng, X.W. (2007) Light-regulated transcriptional networks in higher plants. *Nat. Rev. Genet.*, **8**, 217–230.
- Ma, L., Li, J., Qu, L., Hager, J., Chen, Z., Zhao, H. and Deng, X.W. (2001) Light control of Arabidopsis development entails coordinated regulation of genome expression and cellular pathways. *Plant Cell*, **13**, 2589–2607.
- Oh, E., Yamaguchi, S., Kamiya, Y., Bae, G., Chung, W.I. and Choi, G. (2006) Light activates the degradation of PIL5 protein to promote seed germination through gibberellin in Arabidopsis. *Plant J.*, **47**, 124–139.
- Oh, E., Kim, J., Park, E., Kim, J.I., Kang, C. and Choi, G. (2004) PIL5, a phytochrome-interacting basic helix-loop-helix protein, is a key negative regulator of seed germination in Arabidopsis thaliana. *Plant Cell*, **16**, 3045–3058.
- Shen, H., Moon, J. and Huq, E. (2005) PIF1 is regulated by light-mediated degradation through the ubiquitin-26S proteasome pathway to optimize photomorphogenesis of seedlings in Arabidopsis. *Plant J.*, **44**, 1023–1035.
- Lee, N., Kang, H., Lee, D. and Choi, G. (2014) A histone methyltransferase inhibits seed germination by increasing PIF1 mRNA expression in imbibed seeds. *Plant J.*, **78**, 282–293.
- Oh, E., Yamaguchi, S., Hu, J., Yusuke, J., Jung, B., Paik, I., Lee, H.S., Sun, T.P., Kamiya, Y. and Choi, G. (2007) PIL5, a phytochrome-interacting bHLH protein, regulates gibberellin responsiveness by binding directly to the GAI and RGA promoters in Arabidopsis seeds. *Plant Cell*, **19**, 1192–1208.
- Oh, E., Kang, H., Yamaguchi, S., Park, J., Lee, D., Kamiya, Y. and Choi, G. (2009) Genome-wide analysis of genes targeted by PHYTOCHROME INTERACTING FACTOR 3-LIKE5 during seed germination in Arabidopsis. *Plant Cell*, **21**, 403–419.
- Shi, H., Zhong, S., Mo, X., Liu, N., Nezames, C.D. and Deng, X.W. (2013) HFR1 sequesters PIF1 to govern the transcriptional network underlying light-initiated seed germination in Arabidopsis. *Plant Cell*, **25**, 3770–3784.
- Liu, X., Yang, S., Zhao, M., Luo, M., Yu, C.W., Chen, C.Y., Tai, R. and Wu, K. (2014) Transcriptional repression by histone deacetylases in plants. *Mol. Plant*, **7**, 764–772.
- Brownell, J.E. and Allis, C.D. (1996) Special HATs for special occasions: linking histone acetylation to chromatin assembly and gene activation. *Curr. Opin. Genet. Dev.*, **6**, 176–184.
- Jenuwein, T. and Allis, C.D. (2001) Translating the histone code. *Science*, **293**, 1074–1080.
- Pandey, R., Müller, A., Napoli, C.A., Selinger, D.A., Pikaard, C.S., Richards, E.J., Bender, J., Mount, D.W. and Jorgensen, R.A. (2002) Analysis of histone acetyltransferase and histone deacetylase families of Arabidopsis thaliana suggests functional diversification of chromatin modification among multicellular eukaryotes. *Nucleic Acids Res.*, **30**, 5036–5055.
- Liu, X., Chen, C.Y., Wang, K.C., Luo, M., Tai, R., Yuan, L., Zhao, M., Yang, S., Tian, G., Cui, Y. *et al.* (2013) PHYTOCHROME INTERACTING FACTOR3 associates with the histone deacetylase HDA15 in repression of chlorophyll biosynthesis and photosynthesis in etiolated Arabidopsis seedlings. *Plant Cell*, **25**, 1258–1273.
- Benhamed, M., Bertrand, C., Servet, C. and Zhou, D.X. (2006) Arabidopsis GCN5, HD1, and TAF1/HAF2 interact to regulate histone acetylation required for light-responsive gene expression. *Plant Cell*, **18**, 2893–2903.

25. Charron, J.B., He, H., Elling, A.A. and Deng, X.W. (2009) Dynamic landscapes of four histone modifications during deetiolation in Arabidopsis. *Plant Cell*, **21**, 3732–3748.
26. Zhang, L., Qiu, Z., Hu, Y., Yang, F., Yan, S., Zhao, L., Li, B., He, S., Huang, M., Li, J. *et al.* (2011) ABA treatment of germinating maize seeds induces VP1 gene expression and selective promoter-associated histone acetylation. *Physiol. Plant.*, **143**, 287–296.
27. Tanaka, M., Kikuchi, A. and Kamada, H. (2008) The Arabidopsis histone deacetylases HDA6 and HDA19 contribute to the repression of embryonic properties after germination. *Plant Physiol.*, **146**, 149–161.
28. van Zanten, M., Zöll, C., Wang, Z., Philipp, C., Carles, A., Li, Y., Kornet, N.G., Liu, Y. and Soppe, W.J. (2014) HISTONE DEACETYLASE 9 represses seedling traits in Arabidopsis thaliana dry seeds. *Plant J.*, **80**, 475–488.
29. Clough, S.J. and Bent, A.F. (1998) Floral dip: a simplified method for Agrobacterium-mediated transformation of Arabidopsis thaliana. *Plant J.*, **16**, 735–743.
30. Tang, X., Tian, G., Wang, F., Liu, K., Nguyen, V., Kohalmi, S.E., Keller, W.A., Tsang, E.W., Harada, J.J. *et al.* (2010) Arabidopsis homolog of the yeast TREX-2 mRNA export complex: components and anchoring nucleoporin. *Plant J.*, **61**, 259–270.
31. Perlea, M., Kim, D., Perlea, G.M., Leek, J.T. and Salzberg, S.L. (2016) Transcript-level expression analysis of RNA-seq experiments with HISAT, StringTie and Ballgown. *Nat. Protoc.*, **11**, 1650–1667.
32. Maere, S., Heymans, K. and Kuiper, M. (2005) BiNGO: a Cytoscape plugin to assess overrepresentation of gene ontology categories in biological networks. *Bioinformatics*, **21**, 3448–3449.
33. Huang da, W., Sherman, B.T. and Lempicki, R.A. (2009) Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. *Nat. Protoc.*, **4**, 44–57.
34. Gendrel, A.V., Lippman, Z., Martienssen, R. and Colot, V. (2005) Profiling histone modification patterns in plants using genomic tiling microarrays. *Nat. Methods*, **2**, 213–218.
35. Reed, J.W., Nagpal, P., Poole, D.S., Furuya, M. and Chory, J. (1993) Mutations in the gene for the red/far-red light receptor phytochrome B alter cell elongation and physiological responses throughout Arabidopsis development. *Plant Cell*, **5**, 147–157.
36. Moon, J., Zhu, L., Shen, H. and Huq, E. (2008) PIF1 directly and indirectly regulates chlorophyll biosynthesis to optimize the greening process in Arabidopsis. *Proc. Natl. Acad. Sci. U.S.A.*, **105**, 9433–9438.
37. Yang, X.J. and Seto, E. (2008) The Rpd3/Hda1 family of lysine deacetylases: from bacteria and yeast to mice and men. *Nat. Rev. Mol. Cell Biol.*, **9**, 206–218.
38. Marowa, P., Ding, A. and Kong, Y. (2016) Expansins: roles in plant growth and potential applications in crop improvement. *Plant Cell Rep.*, **35**, 949–965.
39. Xu, P., Cai, X.T., Wang, Y., Xing, L., Chen, Q. and Xiang, C.B. (2014) HDG11 upregulates cell-wall-loosening protein genes to promote root elongation in Arabidopsis. *J. Exp. Bot.*, **65**, 4285–4295.
40. Zhang, Y., Mayba, O., Pfeiffer, A., Shi, H., Tepperman, J.M., Speed, T.P. and Quail, P.H. (2013) A quartet of PIF bHLH factors provides a transcriptionally centered signaling hub that regulates seedling morphogenesis through differential expression-patterning of shared target genes in Arabidopsis. *PLoS Genet.*, **9**, e1003244.
41. Leivar, P. and Quail, P.H. (2011) PIFs: pivotal components in a cellular signaling hub. *Trends Plant Sci.*, **16**, 19–28.
42. Chiwocha, S.D., Cutler, A.J., Abrams, S.R., Ambrose, S.J., Yang, J., Ross, A.R. and Kermod, A.R. (2005) The *etr1-2* mutation in Arabidopsis thaliana affects the abscisic acid, auxin, cytokinin and gibberellin metabolic pathways during maintenance of seed dormancy, moist-chilling and germination. *Plant J. Mol. Biol.*, **42**, 35–48.
43. Debeaujon, I. and Koornneef, M. (2000) Gibberellin requirement for Arabidopsis seed germination is determined both by testa characteristics and embryonic abscisic acid. *Plant Physiol.*, **122**, 415–424.
44. Adkins, S.W. and Ross, J.D. (1981) Studies in Wild Oat Seed Dormancy: I. THE ROLE OF ETHYLENE IN DORMANCY BREAKAGE AND GERMINATION OF WILD OAT SEEDS (*AVENA FATUA* L.). *Plant Physiol.*, **67**, 358–362.
45. Koornneef, M. and van der Veen, J.H. (1980) Induction and analysis of gibberellin sensitive mutants in Arabidopsis thaliana (L.) heynh. *Theor. Appl. Genet.*, **58**, 257–263.
46. Koornneef, M., Bentsink, L. and Hilhorst, H. (2002) Seed dormancy and germination. *Curr. Opin. Plant Biol.*, **5**, 33–36.
47. Leubner-Metzger, G. (2001) Brassinosteroids and gibberellins promote tobacco seed germination by distinct pathways. *Planta*, **213**, 758–763.
48. Steber, C.M. and McCourt, P. (2001) A role for brassinosteroids in germination in Arabidopsis. *Plant Physiol.*, **125**, 763–769.
49. Liu, P.P., Montgomery, T.A., Fahlgren, N., Kasschau, K.D., Nonogaki, H. and Carrington, J.C. (2007) Repression of AUXIN RESPONSE FACTOR10 by microRNA160 is critical for seed germination and post-germination stages. *Plant J.*, **52**, 133–146.
50. Werner, T., Motyka, V., Strnad, M. and Schmulling, T. (2001) Regulation of plant growth by cytokinin. *Proc. Natl. Acad. Sci. U.S.A.*, **98**, 10487–10492.
51. Okamoto, M., Kuwahara, A., Seo, M., Kushiro, T., Asami, T., Hirai, N., Kamiya, Y., Koshihara, T. and Nambara, E. (2006) CYP707A1 and CYP707A2, which encode abscisic acid 8'-hydroxylases, are indispensable for proper control of seed dormancy and germination in Arabidopsis. *Plant Physiol.*, **141**, 97–107.
52. Ma, Y., Szostkiewicz, I., Korte, A., Moes, D., Yang, Y., Christmann, A. and Grill, E. (2009) Regulators of PP2C phosphatase activity function as abscisic acid sensors. *Science*, **324**, 1064–1068.
53. Park, S.Y., Fung, P., Nishimura, N., Jensen, D.R., Fujii, H., Zhao, Y., Lumba, S., Santiago, J., Rodrigues, A., Chow, T.F. *et al.* (2009) Abscisic acid inhibits type 2C protein phosphatases via the PYR/PYL family of START proteins. *Science*, **324**, 1068–1071.
54. Miransari, M. and Smith, D.L. (2014) Plant hormones and seed germination. *Environ. Exp. Bot.*, **99**, 110–121.
55. Oh, E., Zhu, J.Y., Bai, M.Y., Arenhart, R.A., Sun, Y. and Wang, Z.Y. (2014) Cell elongation is regulated through a central circuit of interacting transcription factors in the Arabidopsis hypocotyl. *Elife*, **3**, e03031.
56. Shinomura, T., Nagatani, A., Hanzawa, H., Kubota, M., Watanabe, M. and Furuya, M. (1996) Action spectra for phytochrome A- and B-specific photoinduction of seed germination in Arabidopsis thaliana. *Proc. Natl. Acad. Sci. U.S.A.*, **93**, 8129–8133.
57. Leivar, P. and Monte, E. (2014) PIFs: systems integrators in plant development. *Plant Cell*, **26**, 56–78.
58. Hornitschek, P., Kohnen, M.V., Lorrain, S., Rougemont, J., Ljung, K., López-Vidriero, I., Franco-Zorrilla, J.M., Solano, R., Trevisan, M., Pradervand, S. *et al.* (2012) Phytochrome interacting factors 4 and 5 control seedling growth in changing light conditions by directly controlling auxin signaling. *Plant J.*, **71**, 699–711.
59. Zhang, D., Jing, Y., Jiang, Z. and Lin, R. (2014) The chromatin-remodeling factor PICKLE integrates brassinosteroid and gibberellin signaling during skotomorphogenic growth in Arabidopsis. *Plant Cell*, **26**, 2472–2485.
60. Evans, P.M., Zhang, W., Chen, X., Yang, J., Bhakat, K.K. and Liu, C. (2007) Kruppel-like factor 4 is acetylated by p300 and regulates gene transcription via modulation of histone acetylation. *J. Biol. Chem.*, **282**, 33994–34002.
61. Lee, N., Kang, H., Lee, D. and Choi, G. (2014) A histone methyltransferase inhibits seed germination by increasing PIF1 mRNA expression in imbibed seeds. *Plant J.*, **78**, 282–293.