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RESEARCH PAPER

Arabidopsis thaliana SEED DORMANCY 4-LIKE regulates dormancy and germination by mediating the gibberellin pathway

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Abstract

The molecular mechanisms underlying seed dormancy and germination are not fully understood. Here, we show that *Arabidopsis thaliana* SEED DORMANCY 4-LIKE (AtSdr4L) is a novel specific regulator of dormancy and germination. *AtSdr4L* encodes a protein with an unknown biochemical function that is localized in the nucleus and is expressed specifically in seeds. Loss of function of AtSdr4L results in increased seed dormancy. The germination of freshly harvested seeds of the *Atsdr4l* mutant is insensitive to gibberellin (GA). After-ripened mutant seeds are hypersensitive to the GA biosynthesis-inhibitor paclobutrazol but show unaltered sensitivity to abscisic acid. Several GA biosynthesis genes and GA-regulated cell wall remodeling genes are down-regulated in the mutant in both dormant and after-ripened seeds. These results suggest that the *Atsdr4l* mutation causes both decreased GA biosynthesis and reduced responses. In addition, a genetic analysis indicated that *AtSdr4L* is epistatic to *DELAY OF GERMINATION1* (*DOG1*) for dormancy and acts upstream of *RGA-LIKE 2* (*RGL2*) in the GA pathway. We propose that AtSdr4L regulates seed dormancy and germination by mediating both the DOG1 and GA pathways.

Keywords: Abscisic acid, *Arabidopsis thaliana*, AtSdr4L, DOG1, gibberellin pathway, seed dormancy, seed germination.

Introduction

Seed dormancy is a complex plant fitness trait that delays the timing of germination until the arrival of a favorable season (Bewley, 1997; Finkelstein *et al.*, 2008). Low seed dormancy can lead to premature germination on the mother plant and reduced seed quality. In contrast, high dormancy may lead to non-uniform germination and seedling establishment (Tuan *et al.*, 2018). Dormancy is released by a period of dry storage termed after-ripening and/or by a period of moist chilling termed cold stratification (Graeber *et al.*, 2012; Née *et al.*, 2017*b*). Seed germination requires rupture of the testa followed by rupture of the endosperm. A good understanding of the molecular mechanisms underlying seed dormancy and

the control of germination is important for understanding the ecological behavior of plants and is also of great benefit for agriculture.

The dynamic balance between the plant hormones abscisic acid (ABA) and gibberellin (GA) is a well-known regulatory mechanism for seed dormancy, and plays crucial roles in the induction, maintenance, and release of dormancy (Finch-Savage and Leubner-Metzger, 2006; Razem *et al.*, 2006). ABA positively regulates dormancy, while GA enhances the release of dormancy and the completion of germination. The genes that regulate their metabolism and signaling in seeds are usually involved in the regulation of dormancy and germination. Loss-of-function mutants of ABA biosynthesis genes in Arabidopsis, such as *aba1* (Koornneef *et al.*, 1982), *aba2/3* (Léon-Kloosterziel *et al.*, 1996), and *nced6/9* (Lefebvre *et al.*, 2006), show reduced seed dormancy. In contrast, loss-of-function mutants of ABA catabolism genes such as *CYP707A1* and *CYP707A2* display enhanced seed dormancy (Kushiro *et al.*, 2004; Okamoto *et al.*, 2006). Type 2C protein phosphatases (PP2Cs) are the central regulators in ABA signaling processes. ABA positively regulates seed dormancy by binding PYR/PYL/RCAR receptors and inactivating the activity of PP2C proteins such as ABSCISIC ACID INSENSITIVE1 (ABI1) and ABI2, allowing class II SNF1-RELATED PROTEIN KINASE 2 (SnRK2) to phosphorylate downstream substrates (Ma *et al.*, 2009; Park *et al.*, 2009; Umezawa *et al.*, 2009; Soon *et al.*, 2012).

During seed imbibition, GA promotes endosperm rupture and enhances embryo growth potential by altering the biochemical properties of cell walls (Dekkers *et al.*, 2013; Bassel, 2016). The GA biosynthesis mutants *ga1*, *ga2*, and *ga3* cannot germinate without application of GA (Debeaujon and Koornneef, 2000; Ogawa *et al.*, 2003). Seed dormancy and germination are influenced by GA signaling regulators such as the DELLA protein RGA-LIKE 2 (RGL2) (Lee *et al.*, 2010), the F-box proteins SLEEPY1 (SLY1) and SNEEZY (Ariizumi *et al.*, 2011, 2013), and SPINDLY (SPY) (Swain *et al.*, 2001; Silverstone *et al.*, 2007). RGL2 functions as the major DELLA factor involved in repressing seed germination (Lee *et al.*, 2002; Tyler *et al.*, 2004). RGL2 stimulates ABA biosynthesis and ABI5 activity, and ABA can enhance *RGL2* expression (Lee *et al.*, 2002, 2010; Tyler *et al.*, 2004; Ko *et al.*, 2006; Zentella *et al.*, 2007; Piskurewicz *et al.*, 2008). Thus, RGL2 modulates both the GA and ABA pathways during seed germination.

Epigenetic modification factors have been shown to be involved in the regulation of seed dormancy and germination. H2B monoubiquitination mediated by HISTONE MONOUBIQUITINATION 1 (HUB1) and HUB2 plays a key role in dormancy by regulating dormancy-related genes including those related to ABA metabolism and responses (Liu *et al.*, 2007). A role for histone acetylation and methylation in seed dormancy has been demonstrated by the functional analysis of a number of genes, such as *SIN3-LIKE1* (*SNL1*) and *SNL2* (Wang *et al.*, 2013), *HISTONE DEACETYLASE 9* (*HDA9*) (van Zanten *et al.*, 2014), and *LYSINE SPECIFIC DEMETHYLASE LIKE1* (*LDL1*) and *LDL2* (Zhao *et al.*, 2015). It is interesting to note that the three histone modifications, ubiquitination, acetylation, and methylation, regulate seed dormancy by directly or indirectly influencing the ABA and/or GA pathways, indicating that epigenetic regulation through chromatin modifications is a robust mechanism to alter hormone levels in seeds.

Forward genetics has identified several regulators that are specific to seed dormancy. The main representative members are DELAY OF GERMINATION1 (DOG1) and REDUCED DORMANCY 5 (RDO5). *DOG1* was initially identified as a major quantitative trait locus (QTL) for seed dormancy in Arabidopsis (Bentsink *et al.*, 2006), and it encodes a protein with an unknown biochemical function that is conserved throughout the plant kingdom (Graeber *et al.*, 2014; Huo *et al.*, 2016). DOG1 induces and enhances seed dormancy in response to cold temperatures during seed maturation (Kendall *et al.*, 2011; Nakabayashi *et al.*, 2012). The abundance of the DOG1 protein in freshly harvested seeds is highly correlated with dormancy levels, and hence it has been proposed that it functions as a timer for dormancy release (Nakabayashi *et al.*, 2012). It has been shown that DOG1 mediates a conserved GA-regulated coat dormancy mechanism in a temperature-dependent manner to control seed germination (Graeber *et al.*, 2014), and the mechanism by which it regulates dormancy was recently revealed by its binding to, and inhibition of, the same class of PP2C proteins that are inhibited by ABA (Née *et al.*, 2017*a*; Nishimura *et al.*, 2018). This suggests that the ABA and DOG1 dormancy pathways converge at the PP2C proteins. *RDO5* has been identified as another dormancy-specific gene, and it encodes a pseudophosphatase that binds to DOG1 (Xiang *et al.*, 2014; Née *et al.*, 2017*a*). Additional dormancy regulators have been identified in crop species, such as SEED DORMANCY 4 (Sdr4) in rice (Sugimoto *et al.*, 2010), and MITOGEN-ACTIVATED PROTEIN KINASE KINASE 3 (MKK3) in barley and wheat (Nakamura *et al.*, 2016; Torada *et al.*, 2016). The relationships of these regulators with known dormancy pathways have yet to be determined.

In this study, we report the function of At1g27461 in seed dormancy and germination in Arabidopsis. *At1g27461* is named as *Arabidopsis thaliana SEED DORMANCY 4-LIKE* (*AtSdr4L*) (Subburaj *et al.*, 2016), according to *Sdr4* in rice (Sugimoto *et al.*, 2010). *AtSdr4L* encodes a novel protein with an unknown function. It inhibits dormancy and promotes germination by mediating both GA biosynthesis and its response pathways. Furthermore, AtSdr4L is epistatic to DOG1 for seed dormancy. Thus, we identify a new seed-specific regulator that might integrate the GA and DOG1 pathways in the control of seed dormancy.

Materials and methods

Plant materials and growth conditions

All plants used in this study had the *Arabidopsis thaliana* Columbia (Col) accession background. The *Atsdr4l-1* (SALK_022729) and *Atsdr4l-2* (SALK_203161) mutants were ordered from The Arabidopsis Information Resource (TAIR, https://www.arabidopsis.org/). Homozygous mutant plants were identified by PCR using primers obtained from the Salk Institute Genomic Analysis Laboratory (SIGnAL) database, and were confirmed by quantitative reverse-transcription PCR (qRT-PCR) using RNA isolated from dry and imbibed seeds. The *rgl2-13* mutant was obtained from Nottingham Arabidopsis Stock Centre and was donated by Taiping Sun (Department of Biology,

Duke University, USA; Tyler *et al.*, 2004) and *ga1* (SALK_109115) was a gift from Rongcheng Lin (Institute of Botany, Chinese Academy of Sciences, China; Jiang *et al.*, 2016). Double-mutants were generated by genetic crosses between the single-mutants *Atsdr4l-1* with *dog1-2*, and *Atsdr4l-1* with *rgl2-13*. Plants that were homozygous for both mutations were selected in the F_2 generation. Seed batches were harvested from plants cultivated in soil in a greenhouse at 20±2 °C, 50–60% humidity under long days (16/8 h light/dark). Freshly harvested seeds were either immediately used for analysis or stored at room temperature (25±1 °C) in the dark for at least 6 months to let them after-ripen. All germination comparison assays in this study used seed batches that were harvested at the same time from plants grown in the same environment. The primers used for genotyping are listed in Supplementary Table S1 at *JXB* online.

Dormancy and germination assays

For the seed dormancy assays, ~100 seeds of individual plants were sown on filter paper saturated with sterile distilled water in Petri dishes and cultivated in a growth chamber (16/8 h light/dark, 21±1 °C). For germination assays for GA sensitivity, seeds were freshly harvested, plated onto a filter paper moistened with half-strength Murashige and Skoog (MS) liquid medium (1/2 MS salts, 1% sucrose, 0.025% MES, pH 5.8) supplemented with GA_{4+7} (Sigma-Aldrich), and incubated in the growth chamber. After 7 d, germination was scored according to radicle emergence. For the fluridone treatment, freshly harvested seeds were plated onto wet filter paper moistened with 20 μM fluridone (Sigma-Aldrich). To assess the sensitivity of seeds to ABA and the GA biosynthesis-inhibitor paclobutrazol (PAC), after-ripened seeds were sown on filter paper moistened with half-strength MS liquid medium supplemented with ABA (Fluka) and PAC (Sigma-Aldrich). Following 3 d of stratification (4 °C in darkness), the germination percentages were then determined after 5 d of incubation in the growth chamber at 21±1 °C. For each experiment, at least three biological replicates were performed

Gene expression analysis

Total RNA was extracted from tissues using a Plant Total RNA Purification Kit (GeneMark) and reverse-transcribed using FastQuant RT Kit (TIANGEN). qRT-PCR was carried out with three replicates on an Eppendorf Mastercycler RealPlex real-time system using SYBR® FAST qPCR Master Mix (KAPA Biosystems, Roche) following the manufacturer's instructions. The relative gene expression levels were normalized to those of *ACTIN8* (At1g49240). Each experiment was carried out at least three times with similar results, and one representative result is shown. The primers used for qRT-PCR are listed in Supplementary Table S1.

Plasmid construction

For the $AtSdr4L_{promoter}$*::GUS* construct, a fragment of 1988 bp upstream of the translational start codon was amplified. The pBI101-GUS binary vector was digested first with *Xba*I and *Bam*HI, and then the promoter was cloned into it. To generate cauliflower mosaic virus $35S_{promoter}$*::AtSdr4L-GFP*, the coding sequence (CDS) of *AtSdr4L* without the stop codon was amplified and inserted into the *Bam*HI-*Sal*I site of the pCAMBIA2300-eGFP vector. The $35S_{promoter}$*::YFP-AtSdr4L* construct was made by recombining the AtSdr4L CDS entry clone with pENSG-YFP using Gateway Technology (Invitrogen). All amplified fragments were validated by sequencing. The constructs were electroporated into *Agrobacterium tumefaciens* strain GV3101 or GV3101 with the pMP90RK helper plasmid. The $AtSdr4L_{promoter}$*::GUS*/GV3101 construct was introduced into wild-type Col-0 plants using the floral dip method (Clough and Bent, 1998), and subsequently the transgenic plants were selected on half-strength MS plates supplemented with kanamycin. T_3 homozygous lines with a single insertion were selected for the experiments. The primers used for plasmid construction are listed in Supplementary Table S1.

Subcellular localization

For transient expression in Arabidopsis, protoplast cells were prepared using leaves of 3–4-week-old Arabidopsis seedlings as previously described (Sheen, 2001; Yoo *et al.*, 2007). The $35S_{promoter}$*::AtSdr4L-GFP* and the vector control $35S_{promoter}$*::GFP* were co-transformed with H2B-RFP, a nucleus marker, into protoplasts. After transfection, the protoplasts were cultivated at 22 °C in the dark for 16 h. Cells were subsequently observed under a confocal laser-scanning microscope (Leica TCS SP5). A GFP filter set was used for detection of AtSdr4L specific fluorescence with excitation/emission of 488/509 nm. RFP was visualized to observe the nuclear fluorescence at an excitation wavelength of 585 nm and emission wavelength of 608 nm. Chlorophyll autofluorescence was used as a chloroplast marker. For transient expression in *Nicotiana benthamiana*, the *A. tumefaciens* strain (GV3101/pMP90RK) harbouring a $35S_{promoter}$*::YFP-AtSdr4L* construct was infiltrated into 5–6-week-old *N. benthamiana* leaves. Leaf discs were sampled 2 d after infiltration. The lower epidermis was peeled off and stained with 1 μg ml^{-1} DAPI (Sigma-Aldrich), a nuclei-staining dye. YFP fluorescence and the DAPI staining signal in the epidermal cells were detected using a confocal microscope (Leica TCS SP5).

GUS histochemical analysis

Freshly collected samples from transgenic plants expressing the $AtSdr4L_{promoter}$*::GUS* construct were submerged in a GUS staining solution (Willemsen *et al.*, 1998) and held under vacuum conditions for 15 min, followed by incubation at 37 °C for 3–6 h. After staining, samples were rinsed in 70% ethanol for 1 h before images were captured using a Nikon 80i digital camera.

Phylogenetic analysis of AtSdr4L-related proteins

Amino acid sequences related to AtSdr4L were downloaded from the National Center for Biotechnology Information website (http://blast.ncbi.nlm.nih.gov/). Multiple sequence alignments of these homologs were performed using ClustalX (1.8) and a phylogenetic tree was constructed using the neighbor-joining method in the MEGA6 software.

ABA and GA measurements

For measurement of ABA, ~20 mg dry seeds were used. Quantification was performed using gas chromatography–triple-quadrupole tandem mass spectrometry as described by Müller *et al.*, (2002). For measurement of GA, freshly harvested seeds were imbibed in the growth chamber (16/8 h light/dark, 21±1 °C) for 24 h before sampling. Samples of ~500 mg of imbibed seeds were used. Quantification of GAs was performed using nano-liquid chromatography–electrospray ionization–quadrupole time-of-flight–mass spectrometry (Nano-LC-ESI-Q-TOF-MS) as described previously (Chen *et al.*, 2012; Jiang *et al.*, 2016).

Results

Atsdr4l *mutants show increased seed dormancy*

In order to identify additional genes involved in seed dormancy, we assessed the STRING database (https://string-db.org/) for co-expressed proteins of DOG1, which is a key regulator in dormancy (Bentsink *et al.*, 2006). Among the results, we identified the protein encoded by At1g27461 as the only one that has not been previously studied (Supplementary Fig. S1). This gene has been named as *Arabidopsis thaliana SEED DORMANCY 4-LIKE* (*AtSdr4L*) (Subburaj *et al.*, 2016). An analysis of *AtSdr4L* and *DOG1* using publicly available gene expression data (http://bar.utoronto.ca/efp/cgi-bin/efpWeb.cgi) showed that the two genes have a similar expression pattern

during seed maturation and imbibition (Supplementary Fig. S1).

To examine the function of AtSdr4L in Arabidopsis we studied two mutant alleles, *Atsdr4l-1* (SALK_022729) and *Atsdr4l-2* (SALK_203161), which were obtained from the SALK insertion mutant collection. Both had a transfer DNA (T-DNA) insertion in the single exon of *AtSdr4L* (Fig. 1A). The partial *AtSdr4L* mRNA could not be detected in dry seeds of *Atsdr4l* or in seeds imbibed for 16 h, either by RT-PCR or by qRT-PCR using primer pairs flanking or downstream of the T-DNA insertion, respectively (Fig. 1B, C). In *Atsdr4l-2*, using primers downstream of the T-DNA insertion site, only 10% of the *AtSdr4L* transcript could be detected compared with the wild-type (WT). Thus, *Atsdr4l-1* and *Atsdr4l-2* were identified as loss-of-function mutants of the *AtSdr4L* gene.

The development and morphology of *Atsdr4l* mutant plants were similar to that of the WT under normal growth conditions (Supplementary Fig. S2). As *AtSdr4L* is co-expressed with *DOG1* (Supplementary Fig. S1), we were interested whether AtSdr4L is involved in seed dormancy and therefore we examined the germination of WT and mutant seeds during seed storage. Only 1% of freshly harvested *Atsdr4l* mutant seeds germinated compared with 10% for the WT (Fig. 1D) and during storage the germination rate of the WT increased faster than that of the mutants. After 1 month of dry storage, germination of WT seeds reached 80%, while germination of mutant seeds was still ~10%. The dormancy of *Atsdr4l* seeds stored for 1 month could be completely broken by stratifying them for 3 d at 4 °C (Fig. 1E). Complete dormancy release by after-ripening was reached after 50 d for WT seeds and after 80 d for the mutant seeds (Fig. 1D). These results demonstrated that mutations in *AtSdr4L* caused increased seed dormancy.

AtSdr4L is specifically expressed in seeds and targeted to the nucleus

To further investigate the expression pattern of *AtSdr4L*, we examined its transcript abundance using qRT-PCR and an *AtSdr4L* promoter–β-glucuronidase (GUS) fusion (*AtSdr4L*$_{promoter}$*::GUS*).

qRT-PCR analysis using total RNA prepared from different tissues showed that the *AtSdr4L* transcripts were not detectable

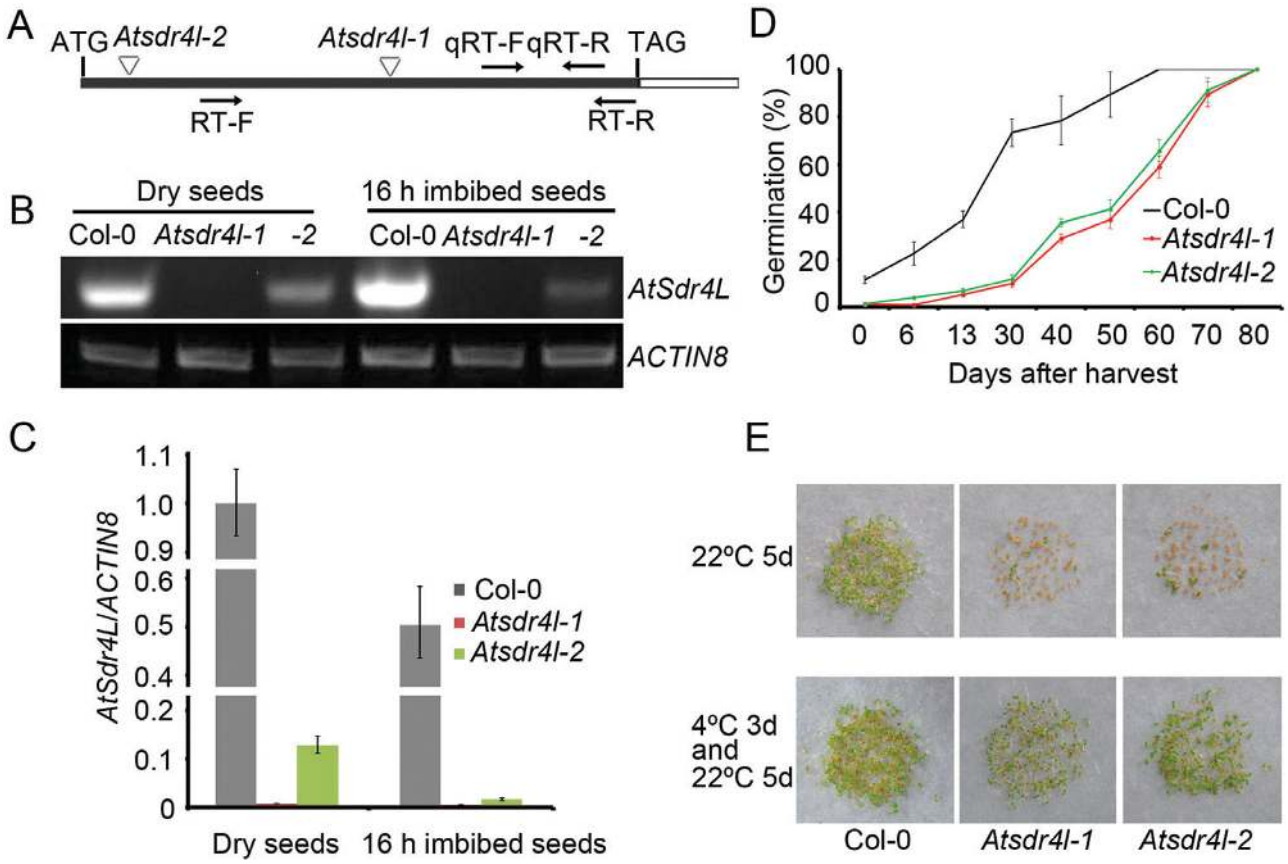


Fig. 1. The *Atsdr4l* mutants show increased seed dormancy. (A) Schematic diagram showing the *AtSdr4L* gene structure and the T-DNA insertion sites in the *Atsdr4l-1* and *Atsdr4l-2* mutant alleles. The *AtSdr4L* coding region is represented in black, the non-coding region in white, and the T-DNA insertions by inverted triangles. The start codon ATG and stop codon TAG are indicated. RT-F/RT-R and qRT-F/qRT-R are the primer pairs used for RT-PCR and qRT-PCR, respectively, and their positions are indicated. (B) RT-PCR analysis of the *AtSdr4L* transcript in the wild-type (Col-0), and *Atsdr4l-1* and *Atsdr4l-2* mutants using the RT-F/RT-R primers. RNA was extracted from dry seeds and seeds imbibed for 16 h. (C) qRT-PCR analysis of the *AtSdr4L* transcript in the wild-type and the mutants using the qRT-F/qRT-R primers. Data are means (±SD) of three independent experiments. (D) Seed germination of Col-0 and the mutants after different periods of dry storage. Data are means (±SD) of at least 10 plants from one experiment. The experiment was performed at least three times with similar results. (E) Dormant *Atsdr4l* seeds at 30 d after harvest were able to germinate after imbibition at 4 °C in darkness for 3 d for stratification. Seeds that had been stored for 30 d were incubated at 22 °C for 5 d on wet filter paper (upper panel), followed by 3 d of stratification and another 5 d at 22 °C (lower panel). (This figure is available in colour at *JXB* online.)

in the roots, stems, leaves, and flowers of seedlings at various ages, or in siliques at 6 d after pollination (DAP) (Fig. 2A). *AtSdr4L* transcripts in siliques increased during seed maturation from 13 DAP, with a maximum in freshly harvested dry seed, after which the transcript levels decreased in after-ripened and imbibed seeds.

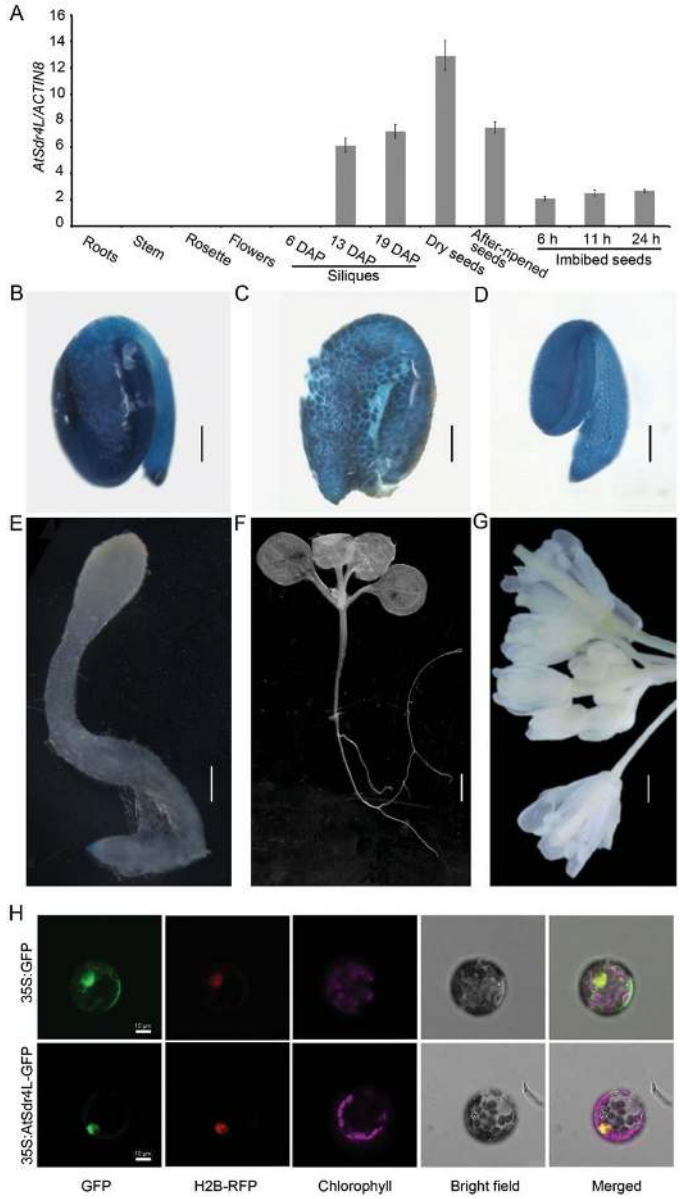


Fig. 2. Expression patterns of *AtSdr4L*. (A) Relative expression of *AtSdr4L* during different plant developmental stages. The tissues and developmental stages tested were: roots of 10-d-old seedlings, stems of 40-d-old plants, rosettes of 20-d-old plants, flower clusters of 40-d-old plants, siliques at 6, 13, and 19 d after pollination (DAP), freshly harvested dry seeds, after-ripened seeds stored for 2 months, and freshly harvested dry seeds imbibed for 6, 11, and 24 h. *ACTIN8* was used as the reference gene. Data are means (±SD), *n*=3. (B–G) Promoter activity of *AtSdr4L* as shown by GUS histochemical staining in *AtSdr4L*$_{promoter}$*::GUS* transgenic plants. (B) Embryo, (C) endosperm, (D) embryo imbibed for 24 h, (E) 2-d-old seedling, (F) 10-d-old seedling, and (G) inflorescence. Scale bars are 100 μm (B–D) and 1 mm (E–G). (H) Nuclear localization of AtSdr4L in Arabidopsis protoplasts. The GFP (upper panel) or AtSdr4L-GFP fusion (lower panel) driven by the 35S promoter was transiently co-expressed with histone H2B-RFP, a nuclear marker. Scale bars are 10 μm. (This figure is available in colour at *JXB* online.)

Consistent with the qRT-PCR data, transgenic Arabidopsis plants expressing the GUS reporter gene driven by the *AtSdr4L* promoter showed strong expression of *AtSdr4L* in the embryo and endosperm of dry seed (Fig. 2B, C). GUS staining was still detected in the embryo after 24 h imbibition (Fig. 2D); however, the GUS signal had disappeared in 2-d old seedlings, apart from a weak signal in the radicle (Fig. 2E). We did not observe GUS staining in 10-d-old seedlings and flowers (Fig. 2F, G). These results supported a seed-specific role for *AtSdr4L*.

Phylogenetic analysis of AtSdr4L and its closest 21 homologs placed dicots and monocots in separate groups (Supplementary Fig. S3). The homologs were uncharacterized proteins, with the exception of *Oryza sativa* Sdr4, which regulates seed dormancy and has been a target of domestication in rice (Sugimoto *et al.*, 2010).

The *AtSdr4L* ORF contains only one exon encoding a predicted protein of 354 amino acids with unknown function. To test the subcellular localization of AtSdr4L, we constructed a fusion between the full-length coding sequence of AtSdr4L and green fluorescent protein (GFP) under the control of the 35S promoter for transient expression. As a positive control, histone H2B was fused to red fluorescent protein (RFP). These constructs were introduced into Arabidopsis leaf mesophyll protoplasts. The AtSdr4L-GFP fusion protein and the positive control H2B-RFP were observed exclusively in the nucleus (Fig. 2H); however, GFP alone was found in both the nucleus and the cytoplasm. This suggested that AtSdr4L is localized to the nucleus. To verify this, a fusion of yellow fluorescent protein (YFP) and AtSdr4L driven by the 35S promoter was constructed for transient expression in *N. benthamiana* leaf epidermal cells. As expected, YFP-AtSdr4L was exclusively localized in the nucleus, and this was confirmed by DAPI staining (Supplementary Fig. S4). These results showed that the AtSdr4L protein is localized in the nucleus.

The dormancy phenotype of Atsdr4l *is epistatic to* dog1

DOG1 is a key regulator of seed dormancy in Arabidopsis (Bentsink *et al.*, 2006), and its expression in freshly harvested seeds correlates with dormancy levels (Nakabayashi *et al.*, 2012). To examine whether the increased seed dormancy phenotype of the *Atsdr4l* mutant was associated with changes in the abundance of *DOG1* transcripts, qRT-PCR assays were performed. During seed maturation from 12 DAP to 16 DAP, *DOG1* transcript levels were slightly lower in the mutants compared to the WT (Fig. 3A), however, levels were higher in the mutants in siliques at 18 DAP, in freshly harvested dry seeds, and in imbibed seeds. We next examined transcript levels of *AtSdr4L* in the *dog1* mutant and found that they were similar to those of the WT in dry seeds and during early imbibition, but levels were lower in seeds imbibed for 24 h (Fig. 3B). The seeds of *dog1-2* are completely non-dormant (Nakabayashi *et al.*, 2012), a phenotype that is the opposite to that of *Atsdr4l*. To study the genetic relationship between *AtSdr4L* and *DOG1*, their double-mutant was generated by crossing and selection. This double-mutant showed enhanced seed dormancy similar to that of the *Atsdr4l* single-mutant (Fig. 3C). Thus, *Atsdr4l*

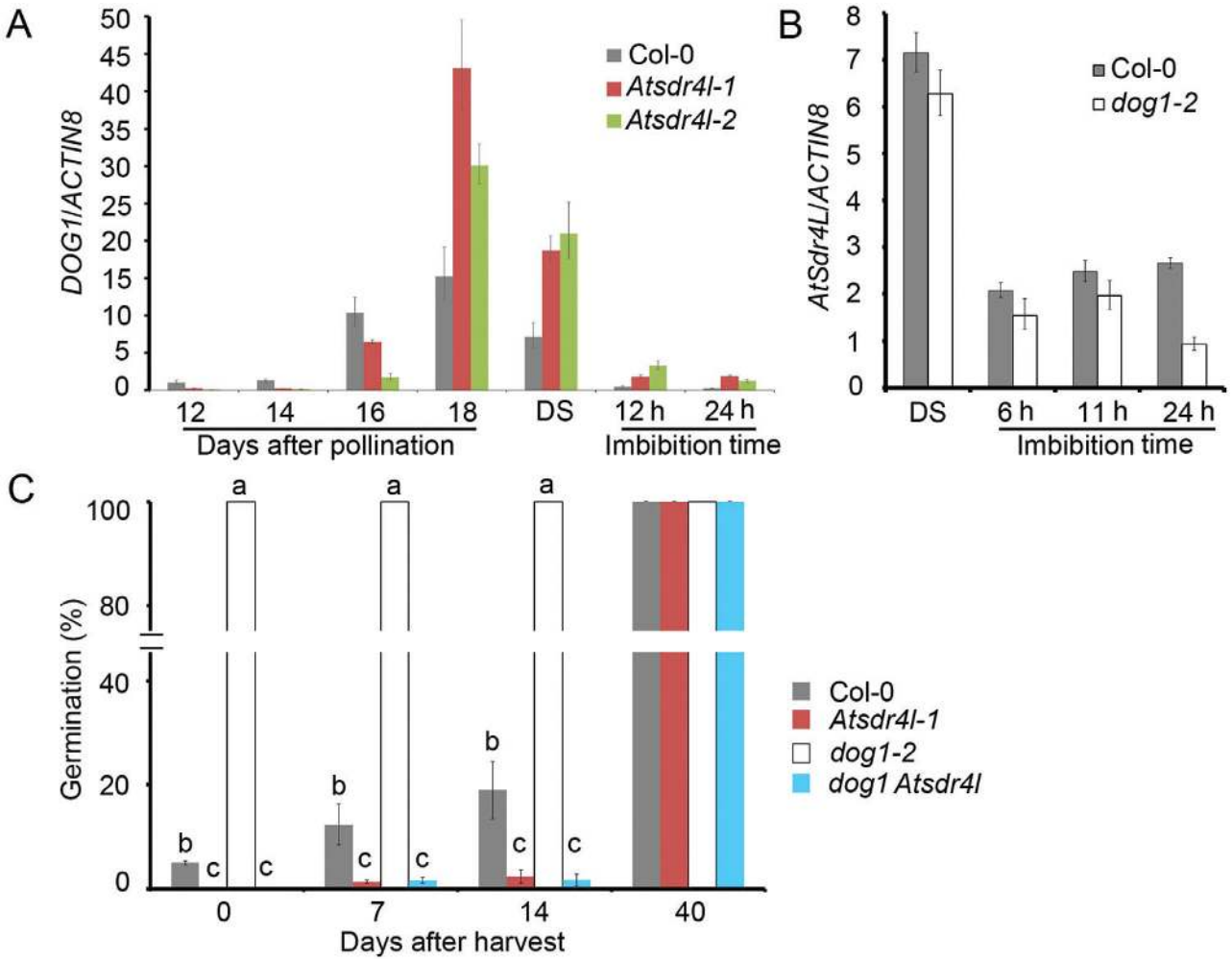


Fig. 3. The *Atsdr4l-1* mutation is epistatic to *dog1* for seed dormancy. (A) qRT-PCR analysis of *DOG1* expression in siliques during seed maturation (12–18 d after pollination) and in freshly harvested dry seeds (DS) and imbibed seeds of the wild-type (Col-0), and the *Atsdr4l-1* and *Atsdr4l-2* mutants. The relative expression was normalized to that of the *ACTIN8* reference gene. (B) Relative expression of *AtSdr4L* in freshly harvested dry seeds and in imbibed seeds of Col-0 and *dog1-2*. *ACTIN8* was used as the reference gene. Data in (A, B) are means (±SD), *n*=3. (C) Germination of Col-0, *Atsdr4l-1*, *dog1-2*, and the *dog1 Atsdr4l* double-mutant after different periods of dry storage. Data are means (±SD) based on eight individual plants for each genotype (~100 seeds per plant). Different letters indicate significant differences according to one-way ANOVA ($P<0.05$). (This figure is available in colour at *JXB* online.)

behaved in an epistatic manner to *dog1* for dormancy, suggesting that *AtSdr4L* functions downstream of *DOG1*.

ABA synthesis is partly involved in the seed dormancy of Atsdr4l

Among the plant hormones, ABA plays a major role in the induction and maintenance of seed dormancy. We therefore attempted to determine whether the enhanced dormancy of *Atsdr4l* was caused by increases in ABA levels or sensitivity. To test this, we examined ABA levels in seeds and the expression of genes involved in ABA metabolism and signaling. As shown in Supplementary Fig. S5, the ABA levels in *Atsdr4l-1* and *Atsdr4l-2* seeds were very similar to those of the WT following after-ripening for 4 weeks, at which point the WT and mutant seeds showed the biggest differences in dormancy phenotype. Only minor differences were detected in the expression of ABA pathway genes between the mutant seeds and the WT, both in freshly harvested and imbibed seeds. In addition, we found that the germination sensitivity to ABA of the mutant seeds was comparable to that of the WT (Supplementary Fig. S5). Interestingly, the ABA biosynthesis inhibitor fluridone reduced the differences in germination between the WT and mutant seeds, but it could not completely break the seed dormancy of *Atsdr4l* (Supplementary Fig. S5). Overall, our data suggested that the dormant phenotype caused by the *Atsdr4l* mutation was at least partly dependent on ABA.

Dormant Atsdr4l *seeds do not germinate when imbibed in GA*

Both the addition of GAs and stratification (chilling of imbibed seeds) have been used to break seed dormancy (Russell *et al.*, 2000; Finch-Savage and Leubner-Metzger, 2006). Treatment of freshly harvested dormant WT seeds with GA_{4+7} resulted in 100% germination, whereas untreated seeds had only 23% germination. However, treatment of *Atsdr4l* seeds with GA_{4+7} did not promote germination, even up to 20 μM (Fig. 4A, C).

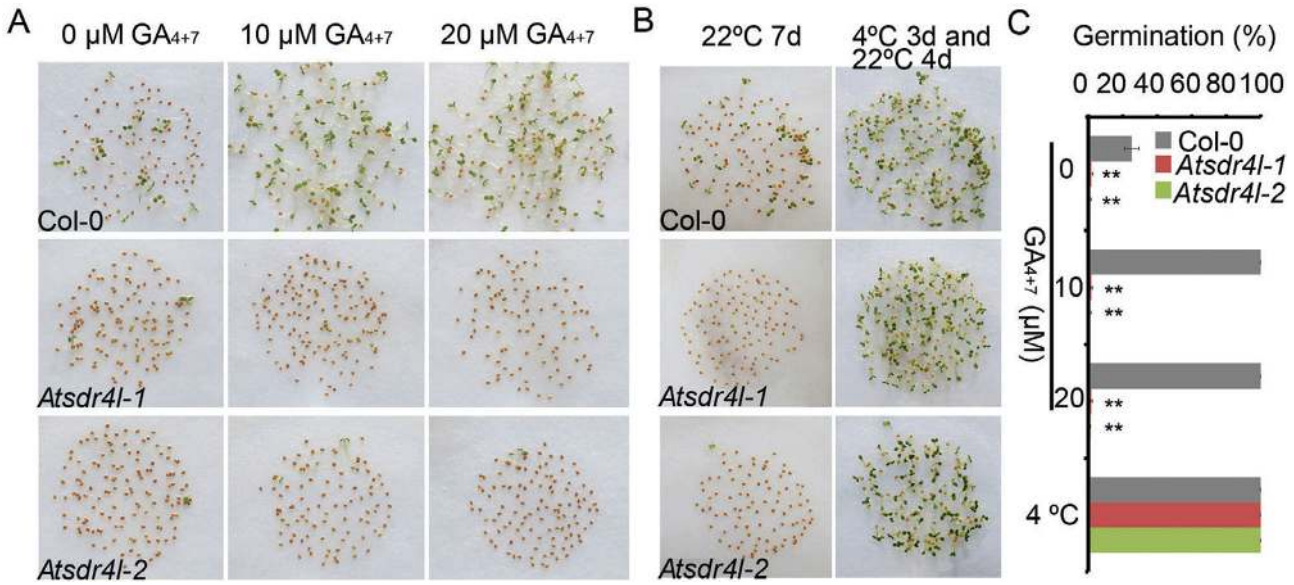


Fig. 4. Treatment with GA does not break the strong dormancy of *Atsdr4l* seeds, but it is broken by stratification. (A) Germination of freshly harvested seeds of the wild-type (Col-0), and the *Atsdr4l-1* and *Atsdr4l-2* mutants imbibed in different GA concentrations. (B) Freshly harvested seeds of Col-0 and the mutants were germinated at 22 °C for 7 d followed by cold stratification, and then transferred to 22 °C for 4 d. (C) Quantification of the germination results shown in (A, B). Data are means (±SD) of at least 100 seeds. Significant differences compared with the wild-type were determined using Student's *t*-test: $^{**}P<0.01$. (This figure is available in colour at *JXB* online.)

In contrast, a stratification treatment was able to release dormancy in the mutant and WT seeds, bringing germination up to 100% in both cases (Fig. 4B, C). Thus, the dormant *Atsdr4l* seeds were viable, but were insensitive to GA application. This indicated that AtSdr4L positively regulates dormancy release through mediation of GA signaling.

Atsdr4l *mutant seeds have decreased GA levels*

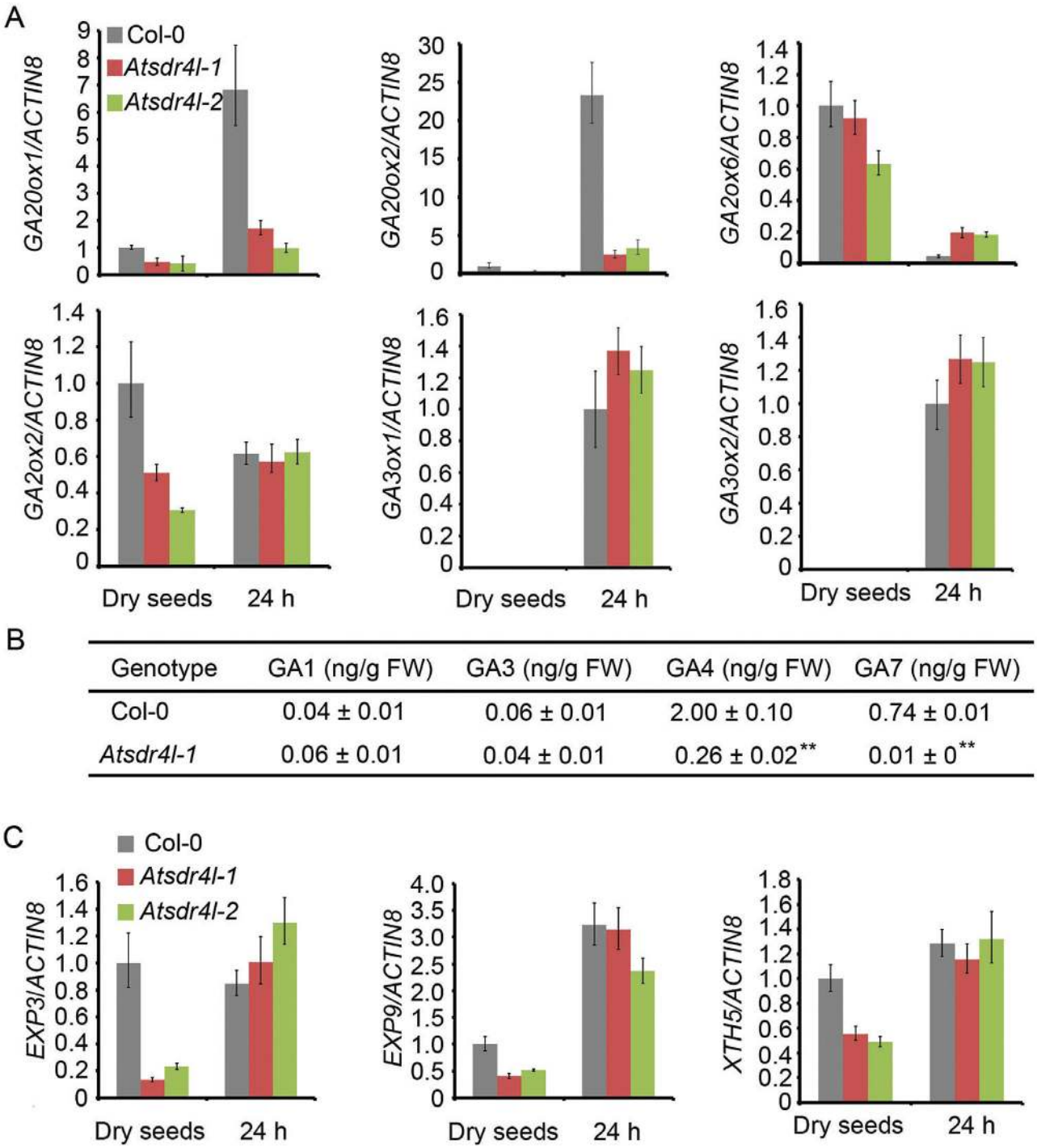
The insensitivity of dormant *Atsdr4l* mutant seeds to GA suggested that this mutant is defective in the response to GA. GA biosynthesis is under negative feedback regulation by the GA response (Olszewski *et al.*, 2002). We therefore speculated that the *Atsdr4l* mutation may increase the expression of GA biosynthesis genes and/or decrease the expression of GA catabolism genes. Intriguingly, however, expression of the GA biosynthesis genes *GA20-oxidase*1 (*GA20ox1*) and *GA20ox2* was significantly down-regulated in the mutant in both dry and imbibed seeds (Fig. 5A). Moreover, the transcript level of the GA catabolic gene *GA2-oxidase6* (*GA2ox6*) was higher in imbibed seeds of the mutants compared to the WT, although dry seeds showed lower *GA2ox2* transcript levels than the wild type. The expression of *GA3-oxidase*1 (*GA3ox1*) and *GA3ox2* was not affected by the *Atsdr4l* mutations in either dry seeds or seeds imbibed for 24 h (Fig. 5A). We also measured endogenous GA levels in freshly harvested seeds of the WT and *Atsdr4l-1* that had been imbibed for 24 h. GA_3 and GA_7 were identified by the mass spectra base peak chromatograms of their standards (Supplementary Fig. S6). In accordance with the lower transcript levels of GA biosynthesis genes, the levels of GA_4 and GA_7 were lower in *Atsdr4l-1* compared with the WT; however, the levels of GA_1 and GA_3 were not altered in the mutant seeds (Fig. 5B). This suggested that the *Atsdr4l* mutation leads to decreased GA biosynthesis during imbibition of freshly harvested seeds. We also examined the expression of GA-response pathway genes, including *MYB65*, *GA-INSENSITIVE DWARF1a* (*GID1a*), *SPINDLY* (*SPY*), and *RGA-LIKE2* (*RGL2*). All of these genes showed similar expression levels in the mutants compared to the WT, in both dry and imbibed seeds (Supplementary Fig. S7).

GA-regulated cell wall remodeling genes are down-regulated in freshly harvested Atsdr4l *seeds*

To confirm the role of AtSdr4L in GA-mediated germination, we examined the expression of GA-regulated genes encoding expansins (EXPs) and xyloglucan endo-transglycosylase/hydrolases (XTHs) (Graeber *et al.*, 2014) in freshly harvested dry and imbibed seeds. These genes are involved in cell wall remodeling and may help to release seed dormancy imposed by the testa and endosperm (Debeaujon and Koornneef, 2000; Yamauchi *et al.*, 2004). We found that *EXP3*, *EXP9*, and *XTH5* were down-regulated in dry seeds of *Atsdr4l-1* and *Atsdr4l-2* compared to the WT (Fig. 5C), although they showed no differences in imbibed seeds. The expression levels of *EXP1* showed no differences compared to the WT. Interestingly, expression of *EXP2* was up-regulated in imbibed seeds of the mutants (Supplementary Fig. S7). These results suggested that AtSdr4L regulates GA-mediated modification of the cell wall.

Germination of Atsdr4l *seeds is hypersensitive to paclobutrazol*

To further determine the role of *AtSdr4L* in GA-mediated germination, we examined the germination phenotype of after-ripened *Atsdr4l* seeds in the presence of the GA biosynthesis-inhibitor paclobutrazol (PAC). We observed higher sensitivity to PAC in the mutant seeds than in the WT



B

Genotype	GA1 (ng/g FW)	GA3 (ng/g FW)	GA4 (ng/g FW)	GA7 (ng/g FW)
Col-0	0.04 ± 0.01	0.06 ± 0.01	2.00 ± 0.10	0.74 ± 0.01
Atsdr4l-1	0.06 ± 0.01	0.04 ± 0.01	0.26 ± 0.02**	0.01 ± 0**

Fig. 5. The *Atsdr4l* mutant has decreased GA biosynthesis. (A) Relative expression of the GA biosynthesis genes *GA20ox1*, *GA20ox2*, *GA3ox1*, and *GA3ox2*, and the GA catabolism genes *GA2ox2* and *GA2ox6* in freshly harvested dry seeds and seeds imbibed for 24 h in the wild-type (Col-0), and the *Atsdr4l-1* and *Atsdr4l-2* mutants. (B) Contents of GA_1, GA_3, GA_4, and GA_7 in freshly harvested seeds imbibed for 24 h for Col-0 and *Atsdr4l-1*. Significant differences compared with the wild-type were determined using Student's *t*-test: $^{**}P<0.01$. (C) Relative expression of the GA-regulated cell wall remodeling genes *EXP3*, *EXP9*, and *XTH5* in freshly harvested dry seeds and seeds imbibed for 24 h for Col-0 and the mutants. All data are means (±SD), $n=3$. (This figure is available in colour at *JXB* online.)

both for stratified seeds (Supplementary Fig. S8) and non-stratified seeds (Supplementary Fig. S9). This hypersensitivity suggested that the *Atsdr4l* mutation causes reduced GA biosynthesis or GA signaling. The mutant seeds did indeed contain reduced amounts of GA_4 and GA_7, but addition of GA to the seeds imbibed with PAC could only partially increase the germination and it was not restored to 100% (Fig. 6). In contrast, WT seeds showed 100% germination when imbibed with a combination of PAC and GA_{4+7}. These results indicated that the PAC-induced reduction in germination in the *Atsdr4l* mutants was due to both reduced GA levels and reduced GA signaling. Hence, AtSdr4L may function as a positive regulator of the GA response and of GA biosynthesis in seed germination.

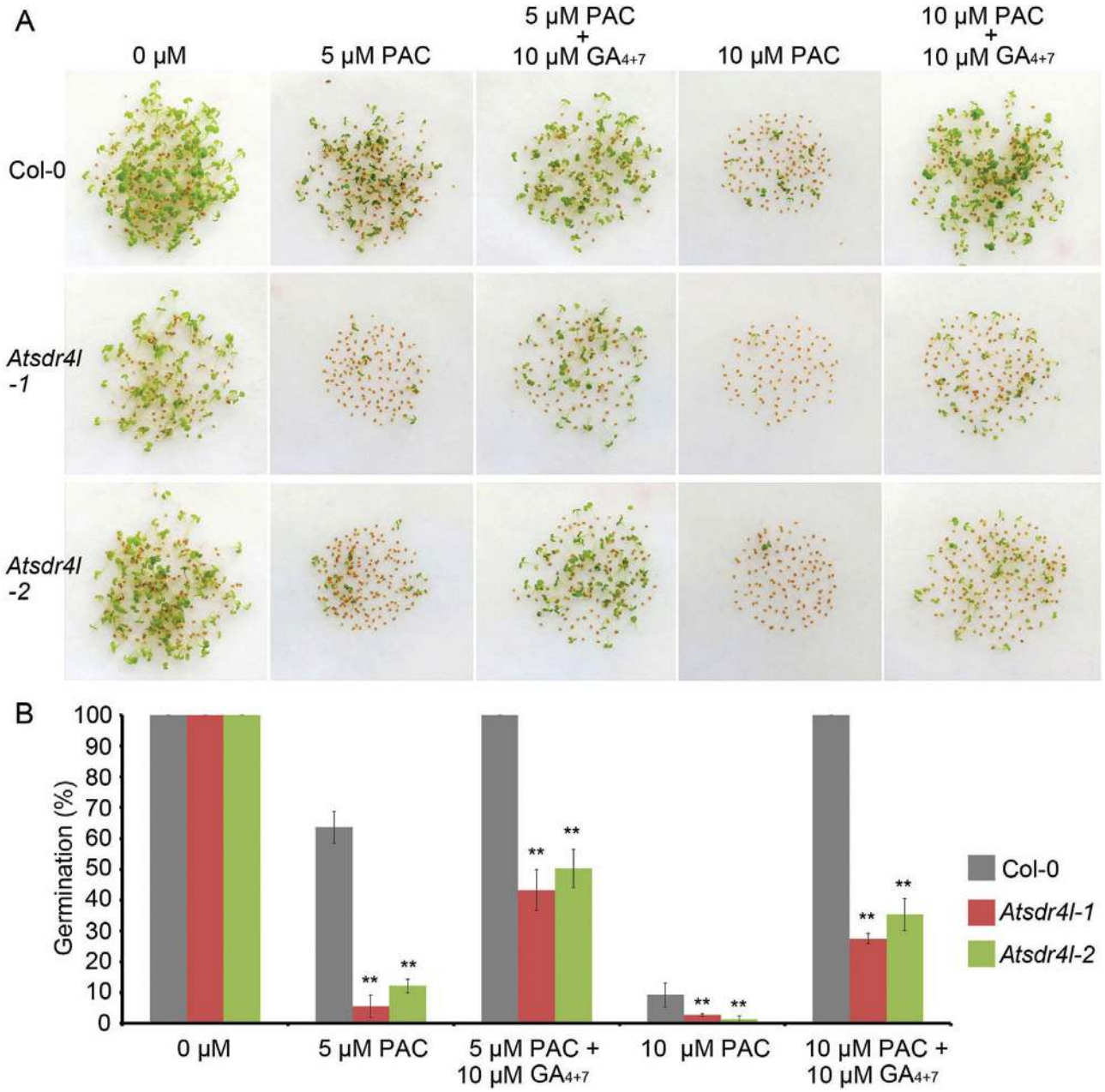


Fig. 6. Germination of after-ripened *Atsdr4l* seeds is hypersensitive to the GA biosynthesis-inhibitor paclobutrazol (PAC). (A) Loss of function of *AtSdr4L* increased the sensitivity of seed germination to PAC. Germination of seeds of the wild-type (Col-0), and the *Atsdr4l-1* and *Atsdr4l-2* mutants was observed 5 d after stratification on wet filter paper containing 0, 5, or 10 μM PAC and supplemented with or without 10 μM GA_{4+7}. (B) Germination of Col-0 and mutant seeds treated with the different concentrations of PAC and supplemented with or without 10 μM GA_{4+7}. Germination was scored 5 d after stratification. Data are means (±SD) of at least 100 seeds. Significant differences compared with the wild-type were determined using Student's *t*-test: $^{**}P<0.01$. (This figure is available in colour at *JXB* online.)

GA biosynthesis and cell wall remodeling genes are down-regulated in after-ripened seeds of Atsdr4l *treated with PAC*

To further confirm a role for AtSdr4L in the promotion of GA levels and GA signaling during seed germination, we examined the effect of the *Atsdr4l* mutation on the expression of GA pathway genes, using after-ripened seeds imbibed with PAC for 12 h. We first examined the expression patterns of GA biosynthesis and catabolism genes. The levels of *GA20ox1* and *GA20ox2* transcripts were lower in mutant seeds treated with PAC compared to the WT (Fig.7A). In contrast, *GA2ox6* expression was up-regulated in the mutant seeds relative to the WT in the PAC treatment. Expression of *GA2ox2* was higher in the mutant compared with the WT in untreated seeds, but the levels were similar when seeds were treated with PAC. The transcript levels of both *GA3ox1* and *GA3ox2* were similar in the mutants and the WT both in the presence and absence of PAC. Expression of *EXP3* and *EXP9* was similar in the mutants and the WT in the absence of PAC, but both genes were down-regulated in the mutants in the presence of PAC (Fig.7B). In contrast the expression of *EXP2* was higher in the mutant seeds without PAC, but showed no differences

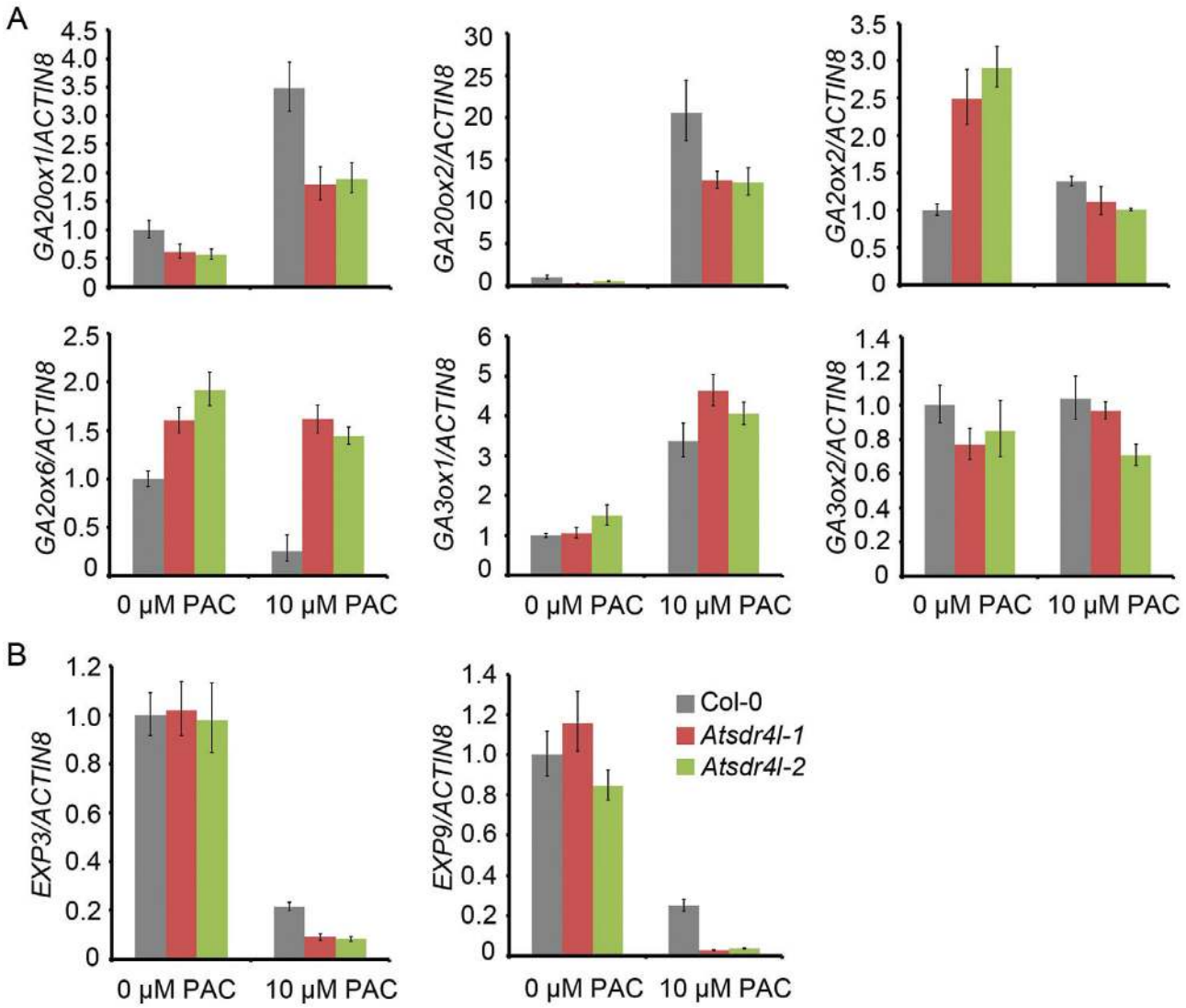


Fig. 7. Expression of GA pathway genes in wild-type (Col-0) and *Atsdr4l* mutant seeds treated with the GA biosynthesis-inhibitor paclobutrazol (PAC). After-ripened seeds were imbibed in 0 μM or 10 μM PAC for 12 h after stratification. (A) Quantitative RT-PCR analysis of the expression levels of selected GA biosynthesis and catabolism genes in Col-0), and the *Atsdr4l-1* and *Atsdr4l-2* mutants. (B) Quantitative RT-PCR analysis of the expression levels of selected GA-regulated genes in Col-0 and the mutants. Expression was normalized to that of the *ACTIN8* reference gene and is presented relative to that of Col-0 with 0 μM PAC treatment, the value of which was set to 1. Data are means (±SD), *n*=3. (This figure is available in colour at *JXB* online.)

with the WT in the presence of PAC (Supplementary Fig. S7). No differences were observed in the expression of *EXP1*, *XTH5*, *RGL2*, *SPY*, and *MYB65*, either with or without PAC treatment (Supplementary Fig. S7). These results supported a positive role for *AtSdr4L* in germination mediated by GA biosynthesis and GA signaling.

AtSdr4L functions upstream of RGL2 in the GA pathway

The DELLA protein RGL2 has a central role in the GA response and is a negative regulator of seed germination that is resistant to PAC (Tyler *et al.*, 2004). To test the genetic interaction between *AtSdr4L* and *RGL2*, the *rgl2-13* and *Atsdr4l-1* mutants were combined by crossing. The resulting double-mutant had a similar sensitivity to PAC as the single-mutant *rgl2-13* (Fig. 8A, B). The *rgl2* mutant shows reduced seed dormancy (Lee *et al.*, 2010), and we also observed reduced dormancy in the *rgl2 Atsdr4l* double-mutant, similar to that of the *rgl2-13* mutant (Fig. 8C). These results indicated that AtSdr4L acts upstream of RGL2.

Discussion

AtSdr4L is a novel specific regulator of Arabidopsis seed dormancy

The control of seed dormancy and germination is complex and our knowledge about its mechanisms is still limited. In this study, we identified AtSdr4L as a novel regulator specific for seed dormancy. AtSdr4L is encoded by At1g27461, which has not been previously analysed. Its protein lacks domains that have a known function. *AtSdr4L* transcription is seed specific (Fig. 2A–G). Its transcripts began to accumulate during seed maturation from 13 d after pollination and reached their highest level in freshly harvested dry seeds. The transcript

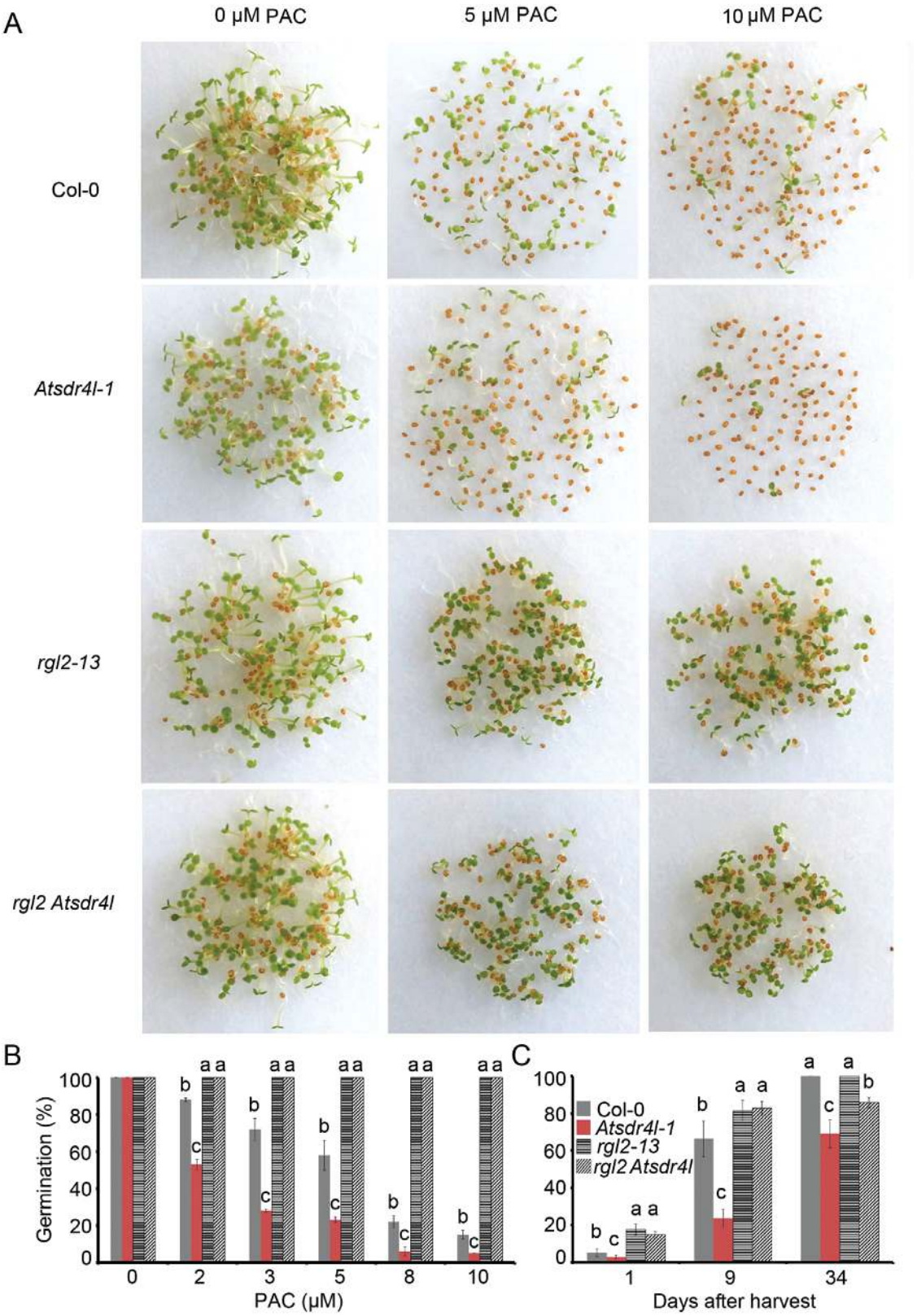


Fig. 8. The germination phenotype of the *rgl2* mutant is epistatic to that of *Atsdr4l*. (A, B) Germination of seeds of the wild-type (Col-0), the *Atsdr4l-1* and *rgl2-13* single-mutants, and the *rgl2 Atsdr4l* double-mutant treated with different concentrations of the GA biosynthesis-inhibitor paclobutrazol (PAC) (0–10 μM). (C) Seed germination of Col-0 and the mutants after different periods of dry storage. Data are means (±SE) of eight individual plants for each genotype (~100 seeds per plant). Different letters indicate significant differences according to one-way ANOVA (P<0.05). (This figure is available in colour at *JXB* online.)

levels decreased during after-ripening and imbibition (Fig. 2A). Interestingly, *AtSdr4L* and *DOG1* had very similar expression patterns (Supplementary Fig. S1). A BLASTP analysis showed that AtSdr4L had highest sequence identity (31%) with rice Sdr4, and *Sdr4* has been identified as a QTL for seed dormancy in rice. *Sdr4* mRNA accumulates during seed maturation, and in mature seed it is expressed throughout the embryo. Sdr4 positively regulates seed dormancy, as loss-of-function mutants of *Sdr4* are completely non-dormant (Sugimoto *et al.*, 2010). The similar expression pattern and high sequence identity between Sdr4 and AtSdr4L suggest that AtSdr4L also has a role in dormancy. In contrast to Sdr4, the *Atsdr4l* T-DNA insertion mutants showed enhanced seed dormancy but they did not show any obvious additional phenotypes (Fig. 1, Supplementary Fig. S2), which suggests that AtSdr4L regulates seed dormancy specifically and negatively. Thus, *AtSdr4L* represents a novel specific regulator for dormancy in Arabidopsis.

AtSdr4L positively regulates GA biosynthesis and GA responses

The balance between ABA and GA integrates environmental signals with other hormone pathways to determine seed dormancy and germination (Holdsworth *et al.*, 2008). Generally, ABA promotes dormancy and dormant seeds have higher ABA levels than non-dormant seeds. Most genes involved in ABA biosynthesis, catabolism, or signaling pathways have a role in seed dormancy, such as *NECD6/9*, *CYP707A1/2*, and *ABI3* (Lefebvre *et al.*, 2006; Okamoto *et al.*, 2006; Sugliani *et al.*, 2009). Surprisingly, our results showed that both the ABA levels in dry seeds and the ABA sensitivity during germination were not affected by the *Atsdr4l* mutation (Supplementary Fig. S5). In addition, the expression levels of key ABA pathway genes including those for biosynthesis and signaling were not altered in the mutants. Interestingly, fluridone treatment was able to promote seed germination of the dormant mutant seeds. These results suggest that the role of AtSdr4L in seed dormancy is partly dependent on the ABA pathway.

GA has been identified as an essential factor for dormancy release and promotion of germination. However, application of GA did not release the dormancy of the *Atsdr4l* mutant seeds, but stratification did (Fig. 4). This suggests that the mutants have defective GA signaling, which could explain the insensitive phenotype of the mutant seeds to GA treatment. This indicates that *AtSdr4L* positively regulates GA-mediated dormancy release. GA biosynthesis is under negative feedback control by the GA signaling pathway. Some of the biosynthesis genes (*GA20ox*s) are subject to negative feedback regulation by the GA response, while *GA2ox* catabolism genes are subject to positive feedback regulation (Olszewski *et al.*, 2002; Sun, 2008). Interestingly, in contrast, expression of *GA20ox1*, *GA20ox2*, and *GA2ox6* did not show regular feedback regulation in freshly harvested *Atsdr4l* seeds, with *GA20ox1* and *GA20ox2* being down-regulated and *GA2ox6* being up-regulated (Fig. 5A). In accordance with this pattern of gene expression, GA levels during imbibition in fresh mature dry seeds were lower in *Atsdr4l* compared with the WT (Fig. 5B). In germinating seeds, the expression of *GA3ox1* but not *GA3ox2* is under negative feedback control by the GA response (Mitchum *et al.*, 2006). Our results showed that transcription of *GA3ox1* and *GA3ox2* were not altered in the GA-insensitive *Atsdr4l* seeds (Fig. 5A). These results demonstrate that most of GA biosynthesis pathway genes that we analysed were not subject to negative feedback control in the mutant seeds with reduced GA signaling, suggesting that AtSdr4L is essential for this feedback control. In accordance with the reduced GA levels, several GA-regulated cell wall-modifying genes are down-regulated in *Atsdr4l* seeds (Fig. 5C), which resulted in delayed germination. Taken together, our data show that AtSdr4L promotes both the GA response and increases GA levels in freshly harvested seeds.

Compared with dry seeds, *AtSdr4L* transcript levels were reduced during seed imbibition, but remain stabled over 24 h of imbibition (Fig. 2A). This indicated that AtSdr4L may have a function during imbibition. We found that after-ripened *Atsdr4l* seeds showed increased sensitivity to inhibition of germination by PAC (Supplementary Figs S8, S9). In addition, adding GA to PAC-treated seeds did not completely rescue the *Atsdr4l* phenotype, although it did increase the germination to some extent (Fig. 6). These results indicated that both the GA response and GA biosynthesis are defective in *Atsdr4l* mutant seeds. In the presence of PAC, the transcript levels of GA-induced *EXP* genes (*EXP3* and *EXP9*) were significantly lower in imbibed after-ripened *Atsdr4l* seeds than in the WT (Fig. 7B). This indicated that GA signaling was decreased in the PAC-treated germinating seeds of the mutant compared with the WT, causing down-regulation of the downstream cell wall-modifying genes and consequently higher sensitivity to PAC. Overall, these data suggest that AtSdr4L promotes germination by positively regulating both GA biosynthesis and the GA response. Interestingly, we found that the transcription of *AtSdr4L* was not induced by GA in *ga1* seeds (Supplementary Fig. S10), indicating that it was not regulated by GA at the transcriptional level.

The DELLA protein RGL2 is the major negative regulator of seed germination mediated by GA signaling (Tyler *et al.*, 2004). GA promotes germination by causing the SCF^{SLY1} (Skp1-Cullin-F-box protein complex) to trigger degradation of RGL2, and hence it disappears before germination (McGinnis *et al.*, 2003; Ariizumi and Steber, 2007). Our genetic analysis of the seeds of the *rgl2 Atsdr4l* double-mutant demonstrated that AtSdr4L acts upstream of RGL2 (Fig. 8). These results also indicate that AtSdr4L is involved in the GA pathway to promote seed germination.

AtSdr4L functions downstream of DOG1

DOG1 is a major QTL for seed dormancy and *dog1* mutants are completely non-dormant (Bentsink *et al.*, 2006). The *dog1-2 Atsdr4l-1* double-mutants showed strongly enhanced seed dormancy that was similar to *Atsdr4l-1* (Fig. 3C). This suggested that AtSdr4L acted downstream of DOG1 to regulate seed dormancy, and that the function of AtSdr4L was suppressed by DOG1. DOG1 did not affect *AtSdr4L* transcript levels in freshly harvested dry seeds or in seeds after 6 h and 11 h of imbibition, as the transcript levels were similar in the seeds of the WT and the *dog1-2* mutant. Furthermore, *AtSdr4L*

was down-regulated in *dog1-2* seeds that had been imbibed for 24 h (Fig. 3B). This indicated that DOG1 did not regulate *AtSdr4L* at the transcript level but probably at the protein level. *DOG1* expression in the *Atsdr4l* mutant seeds was down-regulated during the early and middle stages of maturation (12–16 DAP), while it was up-regulated during late maturation (18 DAP) and in freshly harvested dry seeds and during imbibition (Fig. 3A). This could be feedback regulation as we conclude that AtSdr4L functions downstream of DOG1. *DOG1* transcript abundance is not strictly correlated with its protein accumulation: in freshly harvested seeds transcript levels are low but protein levels are high. (Nakabayashi *et al.*, 2012). It is the abundance of DOG1 protein that is highly correlated with dormancy in freshly harvested seeds (Nakabayashi *et al.*, 2012). The dynamics of the DOG1 protein during seed maturation processes in the *Atsdr4l* seeds therefore need further analysis. We speculate that DOG1 protein levels are higher in *Atsdr4l*, at least in freshly harvested dry seeds. Finally, *DOG1* transcripts quickly disappeared after seed imbibition; however, *AtSdr4L* transcripts were still detectable and remained at the same level during this process (Fig. 3A, B). This indicated that AtSdr4L has additional roles in germination apart from dormancy. Such additional roles would also support a role for AtSdr4L downstream of DOG1.

Taken together, our results indicate that AtSdr4L has a function that is specific to seed dormancy and germination. AtSdr4L inhibits dormancy and promotes germination by regulating the GA pathway downstream of the function of DOG1. Our findings reveal a novel regulator involved in seed germination and hormone signaling. The biochemical function of AtSdr4L is still unknown and further analyses by physical and genetic interaction assays are required.

Supplementary data

Supplementary data are available at *JXB* online.

Fig. S1. *AtSdr4L* is co-expressed with *DOG1*.

Fig. S2. Development and morphology of *Atsdr4l* compared to the wild-type.

Fig. S3. Phylogenetic tree of AtSdr4L and its homologs.

Fig. S4. Subcellular localization of AtSdr4L.

Fig. S5. Effects of *AtSdr4L* mutation on ABA levels and sensitivity in seeds, and response to fluridone treatment.

Fig. S6. Nano-LC-ESI-Q-TOF-MS base peak chromatograms of GA_3 and GA_7.

Fig. S7. Relative expression of the selected GA pathway genes.

Fig. S8. Effects of PAC on germination of stratified *Atsdr4l* seeds.

Fig. S9. Effects of PAC on germination of non-stratified after-ripened *Atsdr4l* seeds.

Fig. S10. Effects of GA on *AtSdr4L* transcript levels.

Table S1. Primer sequences used in this study

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Author contributions: YXL conceived the project; HC and YH performed most of the experiments; JL and MD performed part of the experiments; XL, YL, and FC provided technical assistance; HC and YXL analysed the data; HC wrote the paper; WJJS and YXL contributed to the discussions and modification of the manuscript.

References

Ariizumi T, Hauvermale AL, Nelson SK, Hanada A, Yamaguchi S, Steber CM. 2013. Lifting DELLA repression of *Arabidopsis* seed germination by nonproteolytic gibberellin signaling. Plant Physiology **162**, 2125–2139.

Ariizumi T, Lawrence PK, Steber CM. 2011. The role of two F-box proteins, SLEEPY1 and SNEEZY, in *Arabidopsis* gibberellin signaling. Plant Physiology **155**, 765–775.

Ariizumi T, Steber CM. 2007. Seed germination of GA-insensitive *sleepy1* mutants does not require RGL2 protein disappearance in *Arabidopsis*. The Plant Cell **19**, 791–804.

Bassel GW. 2016. To grow or not to grow? Trends in Plant Science **21**, 498–505.

Bentsink L, Jowett J, Hanhart CJ, Koornneef M. 2006. Cloning of *DOG1*, a quantitative trait locus controlling seed dormancy in *Arabidopsis*. Proceedings of the National Academy of Sciences, USA **103**, 17042–17047.

Bewley JD. 1997. Seed germination and dormancy. The Plant Cell **9**, 1055–1066.

Chen ML, Fu XM, Liu JQ, Ye TT, Hou SY, Huang YQ, Yuan BF, Wu Y, Feng YQ. 2012. Highly sensitive and quantitative profiling of acidic phytohormones using derivatization approach coupled with nano-LC-ESI-Q-TOF-MS analysis. Journal of Chromatography B **905**, 67–74.

Clough SJ, Bent AF. 1998. Floral dip: a simplified method for *Agrobacterium*-mediated transformation of *Arabidopsis thaliana*. The Plant Journal **16**, 735–743.

Debeaujon I, Koornneef M. 2000. Gibberellin requirement for *Arabidopsis* seed germination is determined both by testa characteristics and embryonic abscisic acid. Plant Physiology **122**, 415–424.

Dekkers BJ, Pearce S, van Bolderen-Veldkamp RP, *et al*. 2013. Transcriptional dynamics of two seed compartments with opposing roles in *Arabidopsis* seed germination. Plant Physiology **163**, 205–215.

Finch-Savage WE, Leubner-Metzger G. 2006. Seed dormancy and the control of germination. New Phytologist **171**, 501–523.

Finkelstein R, Reeves W, Ariizumi T, Steber C. 2008. Molecular aspects of seed dormancy. Annual Review of Plant Biology **59**, 387–415.

Graeber K, Linkies A, Steinbrecher T, *et al*. 2014. DELAY OF GERMINATION 1 mediates a conserved coat-dormancy mechanism for the temperature- and gibberellin-dependent control of seed germination. Proceedings of the National Academy of Sciences, USA **111**, E3571–E3580.

Graeber K, Nakabayashi K, Miatton E, Leubner-Metzger G, Soppe WJ. 2012. Molecular mechanisms of seed dormancy. Plant, Cell & Environment **35**, 1769–1786.

Holdsworth MJ, Bentsink L, Soppe WJ. 2008. Molecular networks regulating *Arabidopsis* seed maturation, after-ripening, dormancy and germination. New Phytologist **179**, 33–54.

Huo H, Wei S, Bradford KJ. 2016. DELAY OF GERMINATION1 (DOG1) regulates both seed dormancy and flowering time through microRNA pathways. Proceedings of the National Academy of Sciences, USA **113**, E2199–E2206.

Jiang Z, Xu G, Jing Y, Tang W, Lin R. 2016. Phytochrome B and REVEILLE1/2-mediated signalling controls seed dormancy and germination in *Arabidopsis*. Nature Communications **7**, 12377.

Kendall SL, Hellwege A, Marriot P, Whalley C, Graham IA, Penfield S. 2011. Induction of dormancy in *Arabidopsis* summer annuals requires

parallel regulation of *DOG1* and hormone metabolism by low temperature and CBF transcription factors. The Plant Cell **23**, 2568–2580.

Ko JH, Yang SH, Han KH. 2006. Upregulation of an *Arabidopsis* RING-H2 gene, *XERICO*, confers drought tolerance through increased abscisic acid biosynthesis. The Plant Journal **47**, 343–355.

Koornneef M, Jorna ML, Brinkhorst-van der Swan DL, Karssen CM. 1982. The isolation of abscisic acid (ABA) deficient mutants by selection of induced revertants in non-germinating gibberellin sensitive lines of *Arabidopsis thaliana* (L.) Heynh. Theoretical and Applied Genetics **61**, 385–393.

Kushiro T, Okamoto M, Nakabayashi K, Yamagishi K, Kitamura S, Asami T, Hirai N, Koshiba T, Kamiya Y, Nambara E. 2004. The Arabidopsis cytochrome P450 CYP707A encodes ABA 8′-hydroxylases: key enzymes in ABA catabolism. The EMBO Journal **23**, 1647–1656.

Lee KP, Piskurewicz U, Turecková V, Strnad M, Lopez-Molina L. 2010. A seed coat bedding assay shows that RGL2-dependent release of abscisic acid by the endosperm controls embryo growth in *Arabidopsis* dormant seeds. Proceedings of the National Academy of Sciences, USA **107**, 19108–19113.

Lee S, Cheng H, King KE, Wang W, He Y, Hussain A, Lo J, Harberd NP, Peng J. 2002. Gibberellin regulates *Arabidopsis* seed germination via *RGL2*, a *GAI/RGA*-like gene whose expression is up-regulated following imbibition. Genes & Development **16**, 646–658.

Lefebvre V, North H, Frey A, Sotta B, Seo M, Okamoto M, Nambara E, Marion-Poll A. 2006. Functional analysis of *Arabidopsis NCED6* and *NCED9* genes indicates that ABA synthesized in the endosperm is involved in the induction of seed dormancy. The Plant Journal **45**, 309–319.

Léon-Kloosterziel KM, Gil MA, Ruijs GJ, Jacobsen SE, Olszewski NE, Schwartz SH, Zeevaart JA, Koornneef M. 1996. Isolation and characterization of abscisic acid-deficient *Arabidopsis* mutants at two new loci. The Plant Journal **10**, 655–661.

Liu Y, Koornneef M, Soppe WJ. 2007. The absence of histone H2B monoubiquitination in the *Arabidopsis hub1* (*rdo4*) mutant reveals a role for chromatin remodeling in seed dormancy. The Plant Cell **19**, 433–444.

Ma Y, Szostkiewicz I, Korte A, Moes D, Yang Y, Christmann A, Grill E. 2009. Regulators of PP2C phosphatase activity function as abscisic acid sensors. Science **324**, 1064–1068.

McGinnis KM, Thomas SG, Soule JD, Strader LC, Zale JM, Sun TP, Steber CM. 2003. The *Arabidopsis SLEEPY1* gene encodes a putative F-box subunit of an SCF E3 ubiquitin ligase. The Plant Cell **15**, 1120–1130.

Mitchum MG, Yamaguchi S, Hanada A, Kuwahara A, Yoshioka Y, Kato T, Tabata S, Kamiya Y, Sun TP. 2006. Distinct and overlapping roles of two gibberellin 3-oxidases in *Arabidopsis* development. The Plant Journal **45**, 804–818.

Müller A, Düchting P, Weiler EW. 2002. A multiplex GC-MS/MS technique for the sensitive and quantitative single-run analysis of acidic phytohormones and related compounds, and its application to *Arabidopsis thaliana*. Planta **216**, 44–56.

Nakabayashi K, Bartsch M, Xiang Y, Miatton E, Pellengahr S, Yano R, Seo M, Soppe WJ. 2012. The time required for dormancy release in *Arabidopsis* is determined by DELAY OF GERMINATION1 protein levels in freshly harvested seeds. The Plant Cell **24**, 2826–2838.

Nakamura S, Pourkheirandish M, Morishige H, *et al*. 2016. Mitogen-activated protein kinase kinase 3 regulates seed dormancy in barley. Current Biology **26**, 775–781.

Née G, Kramer K, Nakabayashi K, Yuan B, Xiang Y, Miatton E, Finkemeier I, Soppe WJJ. 2017*a*. DELAY OF GERMINATION1 requires PP2C phosphatases of the ABA signalling pathway to control seed dormancy. Nature Communications **8**, 72.

Née G, Xiang Y, Soppe WJ. 2017*b*. The release of dormancy, a wake-up call for seeds to germinate. Current Opinion in Plant Biology **35**, 8–14.

Nishimura N, Tsuchiya W, Moresco JJ, *et al*. 2018. Control of seed dormancy and germination by DOG1-AHG1 PP2C phosphatase complex via binding to heme. Nature Communications **9**, 2132.

Ogawa M, Hanada A, Yamauchi Y, Kuwahara A, Kamiya Y, Yamaguchi S. 2003. Gibberellin biosynthesis and response during *Arabidopsis* seed germination. The Plant Cell **15**, 1591–1604.

Okamoto M, Kuwahara A, Seo M, Kushiro T, Asami T, Hirai N, Kamiya Y, Koshiba T, 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 Physiology **141**, 97–107.

Olszewski N, Sun TP, Gubler F. 2002. Gibberellin signaling: biosynthesis, catabolism, and response pathways. The Plant Cell **14**, S61–S80.

Park SY, Fung P, Nishimura N, *et al*. 2009. Abscisic acid inhibits type 2C protein phosphatases via the PYR/PYL family of START proteins. Science **324**, 1068–1071.

Piskurewicz U, Jikumaru Y, Kinoshita N, Nambara E, Kamiya Y, Lopez-Molina L. 2008. The gibberellic acid signaling repressor RGL2 inhibits *Arabidopsis* seed germination by stimulating abscisic acid synthesis and ABI5 activity. The Plant Cell **20**, 2729–2745.

Razem FA, Baron K, Hill RD. 2006. Turning on gibberellin and abscisic acid signaling. Current Opinion in Plant Biology **9**, 454–459.

Russell L, Larner V, Kurup S, Bougourd S, Holdsworth M. 2000. The *Arabidopsis* COMATOSE locus regulates germination potential. Development **127**, 3759–3767.

Sheen J. 2001. Signal transduction in maize and *Arabidopsis* mesophyll protoplasts. Plant Physiology **127**, 1466–1475.

Silverstone AL, Tseng TS, Swain SM, Dill A, Jeong SY, Olszewski NE, Sun TP. 2007. Functional analysis of SPINDLY in gibberellin signaling in *Arabidopsis*. Plant Physiology **143**, 987–1000.

Soon FF, Ng LM, Zhou XE, *et al*. 2012. Molecular mimicry regulates ABA signaling by SnRK2 kinases and PP2C phosphatases. Science **335**, 85–88.

Subburaj S, Cao S, Xia X, He Z. 2016. Phylogenetic analysis, lineage-specific expansion and functional divergence of *seed dormancy 4*-like genes in plants. PLoS ONE **11**, e0153717.

Sugimoto K, Takeuchi Y, Ebana K, *et al*. 2010. Molecular cloning of *Sdr4*, a regulator involved in seed dormancy and domestication of rice. Proceedings of the National Academy of Sciences, USA **107**, 5792–5797.

Sugliani M, Rajjou L, Clerkx EJ, Koornneef M, Soppe WJ. 2009. Natural modifiers of seed longevity in the *Arabidopsis* mutants *abscisic acid insensitive3-5* (*abi3-5*) and *leafy cotyledon1-3* (*lec1-3*). New Phytologist **184**, 898–908.

Sun TP. 2008. Gibberellin metabolism, perception and signaling pathways in Arabidopsis. The Arabidopsis Book **6**, e0103.

Swain SM, Tseng TS, Olszewski NE. 2001. Altered expression of *SPINDLY* affects gibberellin response and plant development. Plant Physiology **126**, 1174–1185.

Torada A, Koike M, Ogawa T, Takenouchi Y, Tadamura K, Wu J, Matsumoto T, Kawaura K, Ogihara Y. 2016. A causal gene for seed dormancy on wheat chromosome 4A encodes a MAP kinase kinase. Current Biology **26**, 782–787.

Tuan PA, Kumar R, Rehal PK, Toora PK, Ayele BT. 2018. Molecular mechanisms underlying abscisic acid/gibberellin balance in the control of seed dormancy and germination in cereals. Frontiers in Plant Science **9**, 668.

Tyler L, Thomas SG, Hu J, Dill A, Alonso JM, Ecker JR, Sun TP. 2004. DELLA proteins and gibberellin-regulated seed germination and floral development in *Arabidopsis*. Plant Physiology **135**, 1008–1019.

Umezawa T, Sugiyama N, Mizoguchi M, Hayashi S, Myouga F, Yamaguchi-Shinozaki K, Ishihama Y, Hirayama T, Shinozaki K. 2009. Type 2C protein phosphatases directly regulate abscisic acid-activated protein kinases in Arabidopsis. Proceedings of the National Academy of Sciences, USA **106**, 17588–17593.

van Zanten M, Zöll C, Wang Z, Philipp C, Carles A, Li Y, Kornet NG, Liu Y, Soppe WJ. 2014. HISTONE DEACETYLASE 9 represses seedling traits in *Arabidopsis thaliana* dry seeds. The Plant Journal **80**, 475–488.

Wang Z, Cao H, Sun Y, *et al*. 2013. *Arabidopsis* paired amphipathic helix proteins SNL1 and SNL2 redundantly regulate primary seed dormancy via abscisic acid-ethylene antagonism mediated by histone deacetylation. The Plant Cell **25**, 149–166.

Willemsen V, Wolkenfelt H, de Vrieze G, Weisbeek P, Scheres B. 1998. The *HOBBIT* gene is required for formation of the root meristem in the *Arabidopsis* embryo. Development **125**, 521–531.

Xiang Y, Nakabayashi K, Ding J, He F, Bentsink L, Soppe WJ. 2014. *Reduced Dormancy5* encodes a protein phosphatase 2C that is required for seed dormancy in *Arabidopsis*. The Plant Cell **26**, 4362–4375.

Yamauchi Y, Ogawa M, Kuwahara A, Hanada A, Kamiya Y, Yamaguchi S. 2004. Activation of gibberellin biosynthesis and response pathways by low temperature during imbibition of *Arabidopsis thaliana* seeds. The Plant Cell **16**, 367–378.

Yoo SD, Cho YH, Sheen J. 2007. *Arabidopsis* mesophyll protoplasts: a versatile cell system for transient gene expression analysis. Nature Protocols **2**, 1565–1572.

Zentella R, Zhang ZL, Park M, *et al*. 2007. Global analysis of della direct targets in early gibberellin signaling in *Arabidopsis*. The Plant Cell **19**, 3037–3057.

Zhao M, Yang S, Liu X, Wu K. 2015. *Arabidopsis* histone demethylases LDL1 and LDL2 control primary seed dormancy by regulating *DELAY OF GERMINATION 1* and ABA signaling-related genes. Frontiers in Plant Science **6**, 159.