



ALKBH10B, an mRNA m⁶A Demethylase, Modulates ABA Response During Seed Germination in Arabidopsis

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As the most abundant and reversible chemical modification in eukaryotic mRNA, the epitranscriptomic mark N⁶-methyladenine (m⁶A) regulates plant development and stress response. We have previously characterized that ALKBH10B is an Arabidopsis mRNA m⁶A demethylase and regulates floral transition. However, it is unclear whether ALKBH10B plays a role in abiotic stress response. Here, we found that the expression of *ALKBH10B* is increased in response to abscisic acid (ABA), osmotic, and salt stress. The *alkbh10b* mutants showed hypersensitive to ABA, osmotic, and salt stress during seed germination. Transcriptome analysis revealed that the expression of several ABA response genes is upregulated in *alkbh10b-1* than that of wild type, indicating ALKBH10B negatively affects the ABA signaling. Furthermore, m⁶A sequencing showed that ABA signaling genes, including *PYR1*, *PYL7*, *PYL9*, *ABI1*, and *SnRK2.2* are m⁶A hypermethylated in *alkbh10b-1* after ABA treatment. Taken together, our work demonstrated that ALKBH10B negatively modulates ABA response during seed germination in Arabidopsis.

Keywords: Arabidopsis, N⁶-methyladenine, ALKBH10B, seed germination, ABA, osmotic stress

INTRODUCTION

N⁶-methyladenine (m⁶A) is the most abundant chemical modification in eukaryotic mRNA, and it is dynamically reversible (Jia et al., 2011; Zheng et al., 2013). Different protein complexes (“writer,” “eraser,” and “reader”) can “write,” “erase,” and “recognize” the m⁶A modification in mRNA. In mammals, the “writer” complex of RNA m⁶A mainly consists of three core subunits: METTL3 (methyltransferase like 3), METTL14, and WTAP (Wilms’ tumor 1-associating protein) (Liu et al., 2014; Ping et al., 2014). In addition, the m⁶A modification in RNA can be erased by its “eraser,” such as FTO (fat mass and obesity-associated protein) and ALKBH5 (alkylated DNA repair protein AlkB homolog 5) (Jia et al., 2011; Zheng et al., 2013). As an epitranscriptomic mark, m⁶A modification through its “reader,” such as the YTH family proteins, regulates RNA processing and metabolism, including alternative splicing, mRNA nuclear export, RNA stability maintenance,

translation regulation, and pri-miRNA processing (Fustin et al., 2013; Wang et al., 2014, 2015; Alarcon et al., 2015; Meyer et al., 2015; Xiao et al., 2016).

RNA m⁶A modification is a conservative chemical modification. The mechanisms of “writing,” “erasing,” and “recognizing” of m⁶A in plants are similar to mammals. In *Arabidopsis*, the core subunits of RNA m⁶A methyltransferase complex includes MTA (homolog of human METTL3), MTB (homolog of human METTL14), and FIP37 (homolog of human WTAP). The core subunits of RNA m⁶A methyltransferase are essential proteins for embryonic development (Zhong et al., 2008; Shen et al., 2016; Ruzicka et al., 2017). In rice, the m⁶A methyltransferase are also essential for rice growth and development; mutation of OsFIP causes microspore degradation and affects its fertility (Zhang et al., 2019). There are 13 YTH domain proteins in *Arabidopsis* (Zhou et al., 2018). ECT2/ECT3/ECT4 and CPSF30-L have been confirmed as *Arabidopsis* m⁶A reader proteins. ECT2 promotes stability of m⁶A-modified mRNA and regulates trichome branching (Wei et al., 2018). ECT2/ECT3/ECT4 redundantly regulate the development of *Arabidopsis* epidermis (Arribas-Hernandez et al., 2018; Scutenaire et al., 2018; Wei et al., 2018). CPSF30-L recognizes m⁶A-modified FUE signal to mediate alternative polyadenylation and regulate flowering time, ABA response, and nitrogen metabolism (Hou et al., 2021; Song et al., 2021). The demethylases fine tune RNA m⁶A modification level, thus affect the dynamic state and reversibility of m⁶A. ALKBH9B and ALKBH10B are *Arabidopsis* RNA m⁶A demethylase and play important roles in regulating floral transition and viral infection of alfalfa mosaic virus (AMV). ALKBH10B mediates m⁶A demethylation in *SPL3*, *SPL9*, and *FT* transcripts and regulates their mRNA expression levels through m⁶A-mediated mRNA degradation pathway, thereby affects flowering time (Duan et al., 2017). ALKBH9B is driven to demethylate m⁶A in viral RNA of AMV through the specific interaction with the coat protein of AMV and positively regulates AMV accumulation and systemic invasion (Martinez-Perez et al., 2017). SIALKBH2 is identified as tomato m⁶A demethylase and regulates the ripening of tomato fruit. SIALKBH2 regulates the m⁶A level in the mRNA of DNA 5mC demethylase SIDML2 and affects its expression through m⁶A-mediated mRNA decay (Zhou et al., 2019).

Seed germination is the beginning of a new life cycle in higher plants, which is regulated by internal and environmental signals. The environmental signals perceived by seeds can be incorporated into endogenous hormonal signals. Abscisic acid (ABA) and gibberellic acid (GA) are regarded as the main hormones regulating seed germination (Seo et al., 2006; Shu et al., 2016). ABA positively regulates seed dormancy and inhibits seed germination, while GA acts antagonistically to release dormancy and to initiate seed germination (Shu et al., 2013). In this study, we found ABA, osmotic, and salt stress increase the expression of m⁶A demethylase *ALKBH10B*, which depends on ABI1. Further studies revealed that *ALKBH10B* plays a role in seed germination by negatively affecting ABA response genes. Moreover, we also found several ABA signaling genes with hypermethylated m⁶A peaks in the *alkbh10b* mutant, including *PYR1*, *ABI1*, *SnRK2.2*,

PYL7, and *PYL9*. In all, this study revealed a negative regulatory role of *ALKBH10B* in ABA response during seed germination.

MATERIALS AND METHODS

Plant Materials and Growth Conditions

Arabidopsis thaliana materials used in this study: *alkbh10b-1* (Salk_004215), *alkbh10b-2* (Salk_107289), and *ALKBH10Bp:ALKBH10B/alkbh10b-1* were derived from previous study, all in the Columbia-0 ecotype (Col-0) background (Duan et al., 2017); *abi1-1* (CS22) were in the Landsberg erecta (Ler) background.

All plants were grown at 22°C under long-day conditions (16 h light/8 h dark, white fluorescent tubes, 150–200 μmol m⁻² s⁻¹), and mature seeds of each genotype were collected at the same day, seeds were dried and stored at room temperature. For the seed germination assay, seeds were surfaced sterilized with 20% bleach for 10 min, then rinsed five times with sterile water, and plated on half strength Murashige and Skoog (MS) medium containing 0.8% sucrose, supplemented with various concentrations of ABA (0, 0.5, and 1 μM), 300 mM mannitol, or 150 mM NaCl, respectively. Plates were kept at 4°C in the dark for 3 days, and transferred to a culture room at 22°C under long-day conditions. Radicle emergence was scored after the indicated time intervals.

Quantitative Reverse Transcription-PCR Analysis

Seven-day-old seedlings of Col-0 were used to evaluate *ALKBH10B* expression under external signals treatment. Seven-day-old seedlings were transferred to solid 1/2 MS medium, medium with 300 mM mannitol, 150 mM NaCl, or liquid 1/2 MS medium, medium with 50 μM ABA or 50 μM MeJA, respectively. Seedlings were collected after treatment for different times. Total RNA was isolated with Trizol reagent, and 1 μg total RNA was used for reverse transcription by PrimeScript RT reagent kit with gDNA Eraser (Takara). qRT-PCR was performed using SYBR Green Master Mix (YEASEN) on a ViiA 7 Dx (Applied Biosystems). All qRT-PCRs were performed in triplicate using total RNA samples extracted from three independent biological replicates. The 2^{-ΔΔCT} method was used to calculate the gene expression levels. *At2G28390* was used as an internal control (Czechowski et al., 2005). All primers used are listed in **Supplementary Table 2**.

m⁶A Sequencing

Seven-day-old seedlings of *alkbh10b-1* and Col-0 were treated with half strength liquid MS medium supplemented with 50 μM ABA for 3 h, and the total RNA were extracted. Poly (A)⁺ RNA was isolated from total RNA using oligo(dT)₂₅ Dynabeads (Thermo Fisher Scientific). 5 μg poly(A)⁺ RNA was fragmented into 100–150 nt by Magnesium RNA fragmentation module (NEB). m⁶A immunoprecipitation and library preparation were performed using the EpiMark N⁶-Methyladenosine enrichment kit (NEB). Input and RNA eluted from m⁶A-IP were used to prepare libraries with NEBNext Ultra II RNA Library Prep Kit.

Sequencing was performed on an Illumina HiSeq X Ten machine in pair-end mode with 150 bp per read (Genewiz).

Analysis of m⁶A-Seq Data

Sequence data were analyzed according to the procedure described by Song et al. (2021). Briefly, adapters and low-quality reads were trimmed by Cutadapt (v1.18) (Martin, 2011), clean reads were mapped to TAIR10 by HISAT2 (v2.1.0) (Pertea et al., 2016). PCR duplication were filtered by Picard Toolkit. The m⁶A peaks were identified using the MACS2 (Zhang et al., 2008) peak-calling algorithm based on enrichment criteria (IP/Input) ≥ 2 and FDR < 0.05 . Hypermethylated m⁶A sites were identified by MeTDiff (Cui et al., 2018) based on enrichment criteria fold change ≥ 2 and FDR < 0.05 . To evaluate the m⁶A distribution in transcripts, we divided TAIR10 transcripts into five-non-overlapping regions: 5'UTRs,

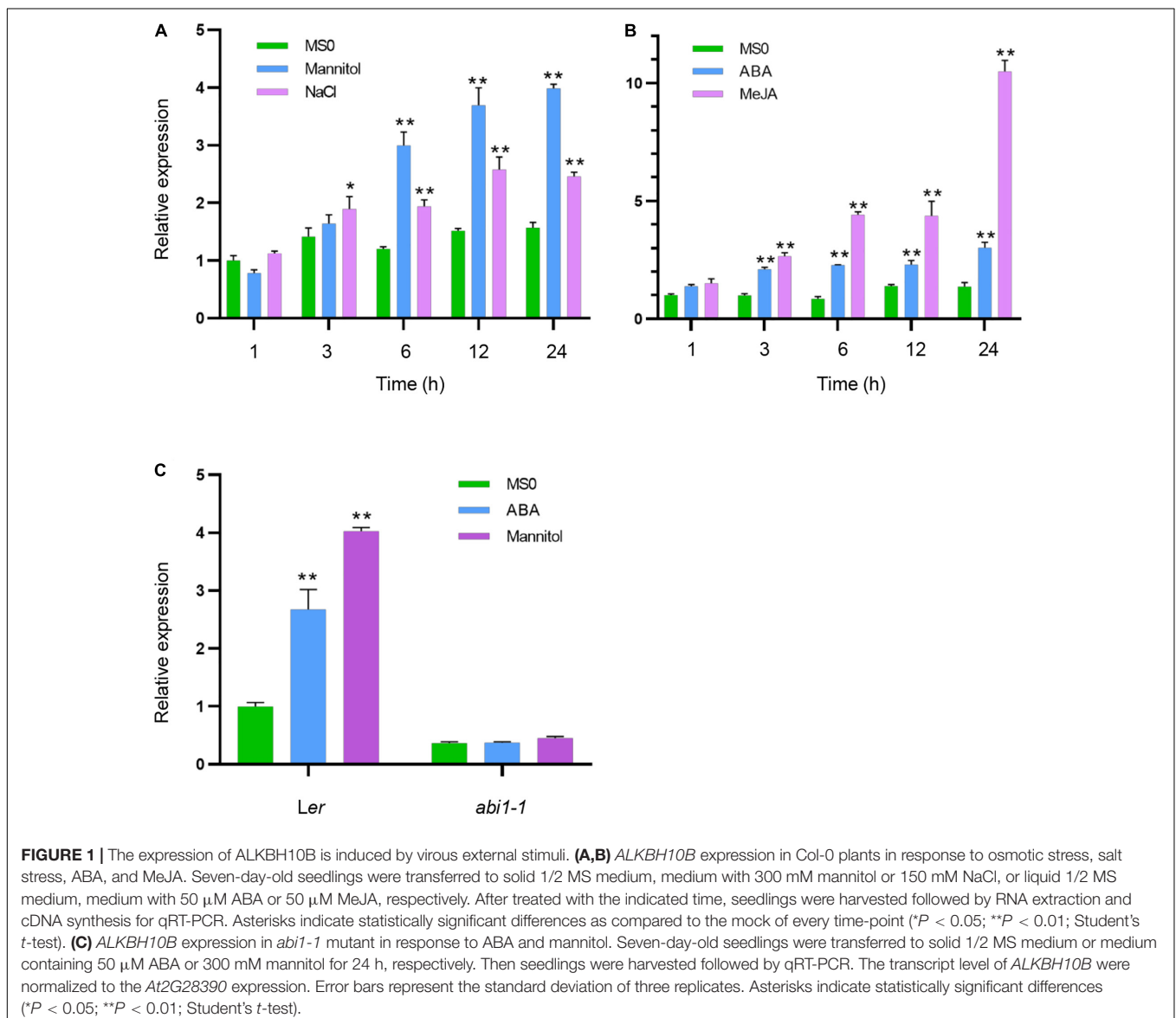
Start_Codon (100-nucleotide window centered on the start codon), coding sequences (CDSs), Stop_Codon (100-nucleotide window centered on the stop codon) and 3'UTRs (Duan et al., 2017). We used a python script to assert subtypes of those RNA molecules contain hypermethylated m⁶A peak (scripts can be found in Github¹). Candidate gene list was submitted to DAVID² to perform GO ontology (GO) and KEGG pathway enrichment analyses.

Analysis of RNA-seq Data

The input data of m⁶A sequencing was used for transcriptome data analysis. Sequencing reads were trimmed and mapped to the reference genome (TAIR10) by Cutadapt (v1.18) and HISAT2 (v2.1.0), respectively. The differential expression genes

¹https://github.com/joybio/m6A-seq/tree/main/feature_annotation/

²<https://david.ncifcrf.gov/tools.jsp>



(DEG) were identified by R package (DESeq2) (Love et al., 2014) with a cutoff criterion of fold change ≥ 1.5 and FDR < 0.05 . Gene ontology (GO) enrichment analyses were performed by using DAVID.

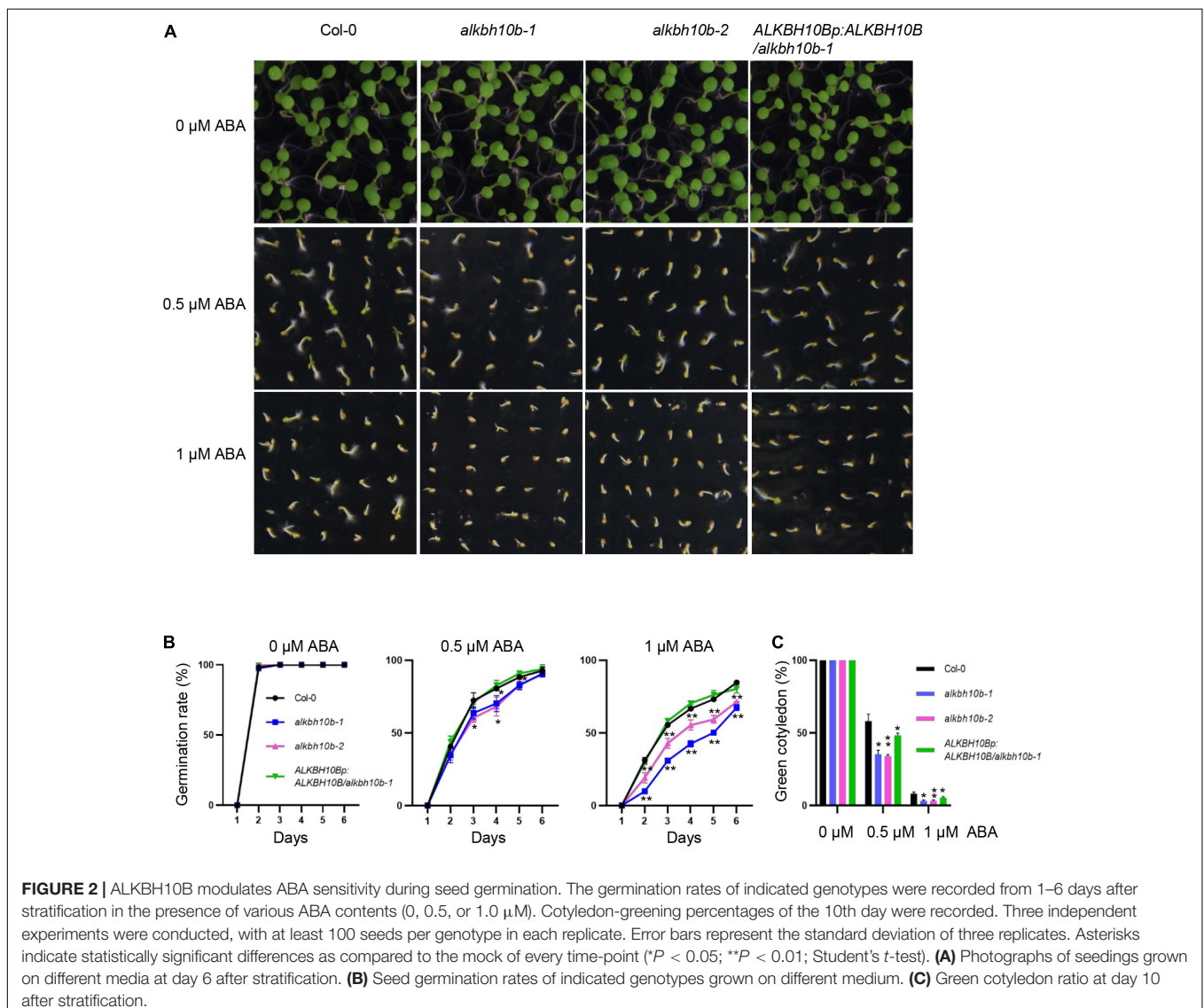
RESULTS

ALKBH10B Expression is Induced by ABA, JA, Osmotic, and Salt Stress

In our previous study, we have identified ALKBH10B as an mRNA m⁶A demethylase, which regulates floral transition in Arabidopsis (Duan et al., 2017). In addition to developmental function of ALKBH10B, we asked whether ALKBH10B is involved in abiotic stress response. We firstly examined whether the expression level of *ALKBH10B* is affected by osmotic and salt stress. Seven-day-old seedlings growing on 1/2 MS medium were transferred to solid medium containing 300 mM

mannitol or 150 mM NaCl. Seedlings were collected after time-course treatment (1, 3, 6, 12, or 24 h). After treatment with mannitol for 6 h, or NaCl for 3 h, the expression of *ALKBH10B* was significantly increased than that of the control (Figure 1A). This is consistent with the data in AtGenExpress global stress expression database (Kilian et al., 2007). Both ABA and jasmonic acid (JA) are important phytohormones which regulate environment response (Ju et al., 2019). Next, we tested whether *ALKBH10B* expression is activated by ABA and methyl jasmonate (MeJA) treatment. The results showed that after treatment with ABA or MeJA for 3 h, the expression of *ALKBH10B* was significantly increased (Figure 1B).

In ABA signaling pathway, ABA is perceived by the intracellular pyrabactin resistance 1 (PYR1) and PYR1-like (PYL)/regulatory component of ABA receptor (RCAR) proteins. From the transcriptome data of previous studies (Zhao et al., 2018), in *pyl* duodecuple mutant, *pyr1pyl1/2/3/4/5/7/8/9/10/11/12*, the induction of *ALKBH10B*



expression by ABA or mannitol was much less in the mutant (**Supplementary Figure 1**). ABA insensitive 1 (ABI1), encodes group A protein phosphatase 2Cs (PP2C), which regarded as ABA co-receptor (Leung et al., 1994), is crucial for ABA signaling. The activation of *ALKBH10B* expression by ABA or mannitol was blocked in the *abi1-1* mutant (**Figure 1C**). These results indicated that the expression of *ALKBH10B* is regulated by the PYR/PYL/RCAR-ABI1 mediated ABA signaling pathway.

***ALKBH10B* Modulates Seed Germination and Post-germination Development Responding to ABA, Salt, and Osmotic Stress**

The expression of *ALKBH10B* is activated by ABA, suggesting that *ALKBH10B* plays potential roles in ABA response in Arabidopsis. To understand the roles of *ALKBH10B* in

ABA response, two T-DNA insertion mutants: SALK_004215 (*alkbh10b-1*) and SALK_107289 (*alkbh10b-2*), as well as one complementation material (*ALKBH10Bp:ALKBH10B/alkbh10b-1*) arrived from our previous study were used in the following research. Seed germination is sensitive to ABA concentration; Therefore, we performed the seed germination assay of each genotype under ABA treatment. We germinated the seeds of *alkbh10b-1*, *alkbh10b-2*, *ALKBH10Bp:ALKBH10B/alkbh10b-1*, and wild type (Col-0) on 1/2 MS medium with or without ABA treatment. In the absence of ABA, the germination rate of different genotypes was similar (**Figures 2A,B**). In the presence of different concentrations of ABA, *alkbh10b-1* and *alkbh10b-2* seeds were more sensitive to ABA inhibition, both mutants showed lower germination rate compared to the wild type (**Figures 2A,B**). Complementation of *ALKBH10B* in *alkbh10b-1* (*ALKBH10Bp:ALKBH10B/alkbh10b-1*) recovered the lower germination rate of *alkbh10b-1* under ABA treatment

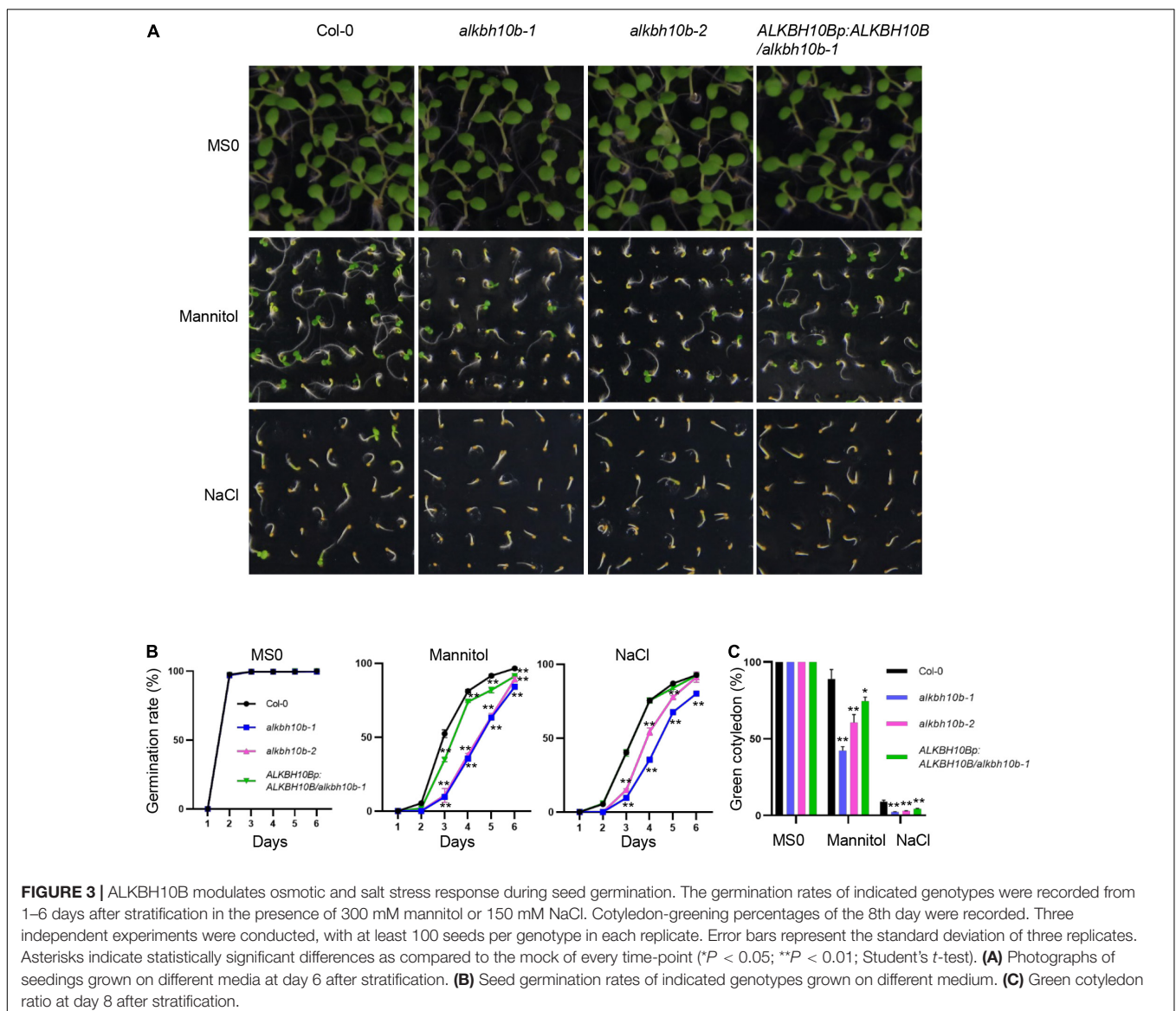
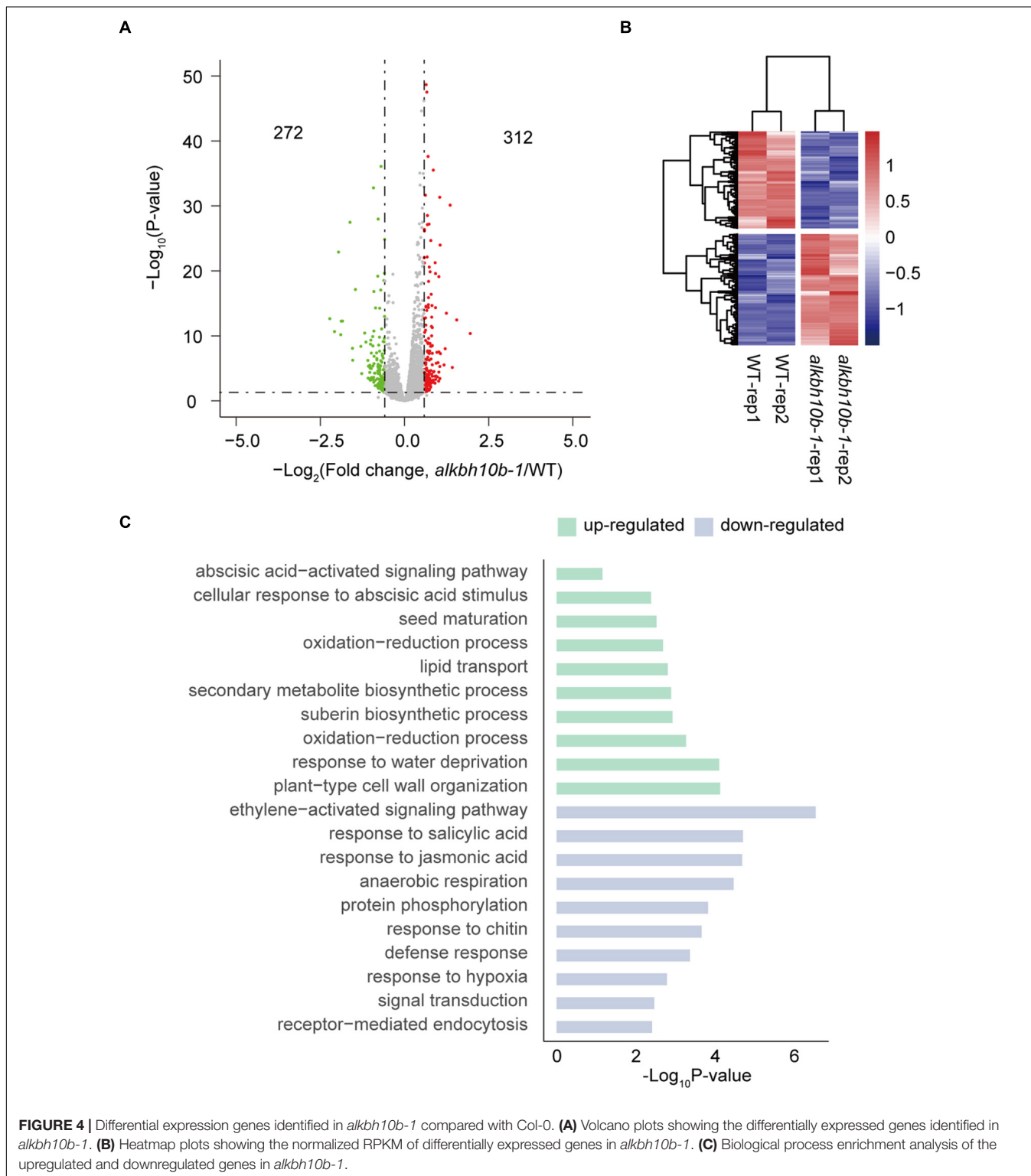


FIGURE 3 | *ALKBH10B* modulates osmotic and salt stress response during seed germination. The germination rates of indicated genotypes were recorded from 1–6 days after stratification in the presence of 300 mM mannitol or 150 mM NaCl. Cotyledon-greening percentages of the 8th day were recorded. Three independent experiments were conducted, with at least 100 seeds per genotype in each replicate. Error bars represent the standard deviation of three replicates. Asterisks indicate statistically significant differences as compared to the mock of every time-point (* $P < 0.05$; ** $P < 0.01$; Student's t -test). **(A)** Photographs of seedlings grown on different media at day 6 after stratification. **(B)** Seed germination rates of indicated genotypes grown on different medium. **(C)** Green cotyledon ratio at day 8 after stratification.

(Figures 2A,B). In line with the germination rate data, *alkbh10b-1* and *alkbh10b-2* mutants also showed lower cotyledon greening rates after 10 days of germination (Figure 2C). These results indicated that ALKBH10B acts as a negative regulator of ABA signaling during seed germination and post-germination growth.

We also tested whether ALKBH10B affects the seed germination in the presence of osmotic and salt stress. The seeds were sown on 1/2 MS medium supplemented with 300 mM mannitol or 150 mM NaCl. Compared with the wild type seeds, germination and cotyledon greening ratios of *alkbh10b-1* and



alkbh10b-2 seeds were more inhibited under mannitol or NaCl treatment (Figures 3A–C), indicating that ALKBH10B plays roles in regulating seed germination and post-germination growth in response to osmotic and salt stress.

Transcriptome Analysis of *alkbh10b-1* After ABA Treatment

Since both *alkbh10b* mutants are more sensitive to ABA than Col-0, we speculated that ALKBH10B may affect the expression of some ABA response genes. Therefore, we performed RNA sequencing experiments to profile the transcriptome of *alkbh10b-1* and the wild type after ABA treatment. Total RNA was isolated from 7-day-old seedlings treated with ABA for 3 h and used for RNA-seq library construction. We identified 312 upregulated genes and 272 downregulated genes (fold change ≥ 1.5 and FDR ≤ 0.05) (Figure 4A and Supplementary Dataset 2). Hierarchical clustering depicted differential expression profiles in all samples (Figure 4B). Then we performed GO analysis of these DEG using DAVID. The results showed that the upregulated genes were significantly enriched in cellular response to ABA stimulus, ABA activated signaling pathway, seed maturation and water deprivation (Figure 4C), which suggest that ALKBH10B may participate in ABA signaling pathway by affecting genes involved in ABA signaling pathway and plays a role in seed germination, which corresponds to the phenotype of *alkbh10b* mutation lines. Furthermore, downregulated genes were significantly enriched in the response of plant hormone signaling, including salicylic acid and JA (Figure 4C), which are important in various stress response and signal transduction. These results suggest ALKBH10B plays a role in abiotic stress response and system defense.

ALKBH10B Negatively Affects the Expression of ABA Response Genes

RNA-seq data revealed that the upregulated genes were significantly enriched in ABA response and seed maturation, which may explain why the *alkbh10b* mutants were more sensitive under ABA treatment. *ABI3* and *ABI5* are two important transcription factors in regulating seed maturation, germination, and early development of seedlings (Bensmihen et al., 2002; Lopez-Molina et al., 2002). We measured the transcript levels of *ABI3*, *ABI5*, and some of their downstream genes, including *RD29B*, *EM6*, and *EM1* in Col-0 and *alkbh10b-1* using qRT-PCR under mock and ABA treatment. The results showed that the expression of *ABI3*, *ABI5*, *EM6*, *EM1*, and *RD29B* were upregulated in *alkbh10b-1* after ABA treatment (Figure 5). Among them, the higher expression of *EM6* and *RD29B* in *alkbh10b-1* was consistent with the RNA-seq data; the higher expression of *ABI3*, *ABI5*, and *EM1* were not identified in the RNA-Seq data because of their low expression or their insignificant difference between *alkbh10b-1* and wild type. To better understand the role of ALKBH10B in seed germination, we detected the expression patterns of these ABA signaling-related genes in seeds germinated for 2.5 days on medium containing 0 or 0.5 μM ABA. The results showed that the expression of *ABI3*, *ABI5*, *EM6*, *EM1*, and *RD29B* in *alkbh10b-1* was higher than that

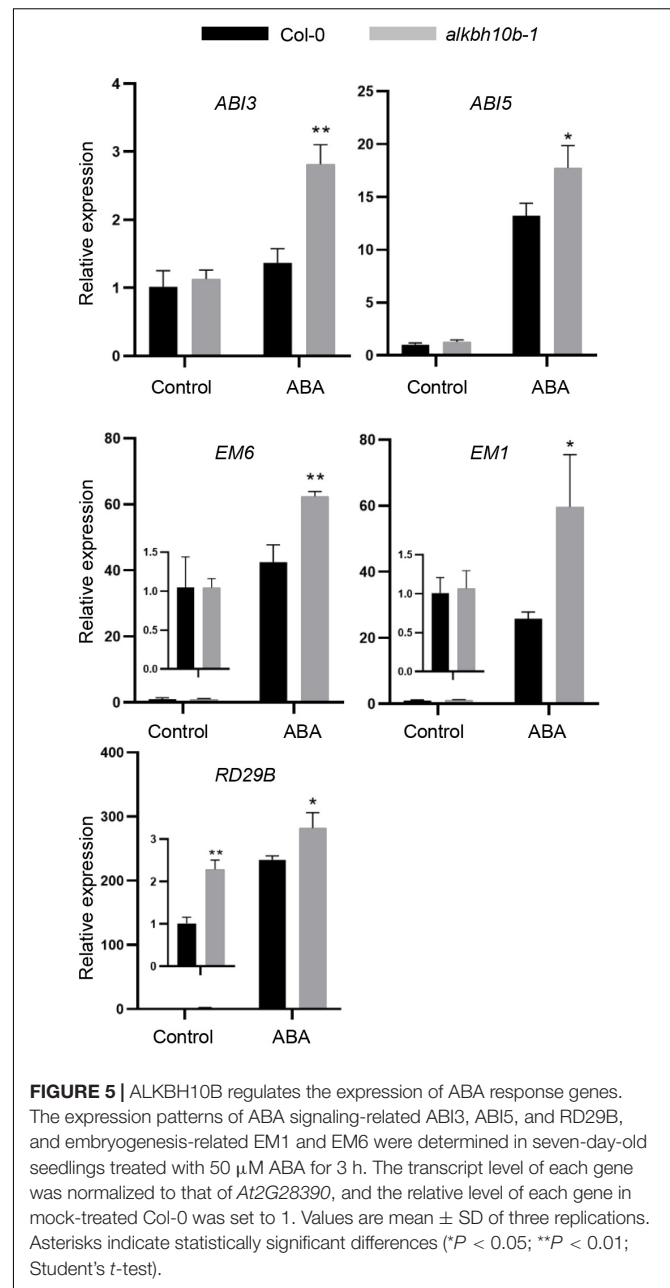
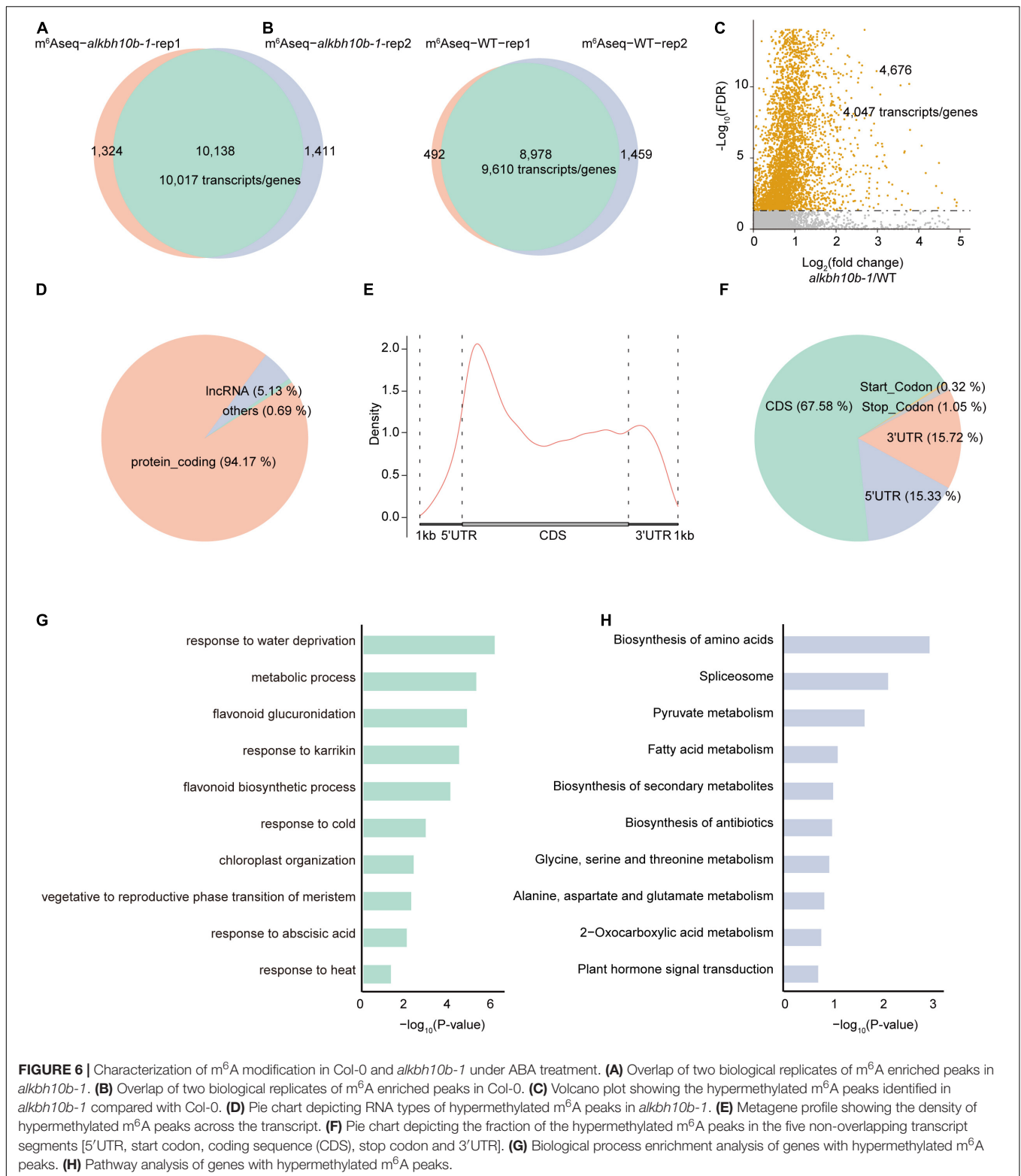


FIGURE 5 | ALKBH10B regulates the expression of ABA response genes. The expression patterns of ABA signaling-related *ABI3*, *ABI5*, and *RD29B*, and embryogenesis-related *EM1* and *EM6* were determined in seven-day-old seedlings treated with 50 μM ABA for 3 h. The transcript level of each gene was normalized to that of *At2G28390*, and the relative level of each gene in mock-treated Col-0 was set to 1. Values are mean \pm SD of three replications. Asterisks indicate statistically significant differences (* $P < 0.05$; ** $P < 0.01$; Student's *t*-test).

of the Col-0 on medium with or without ABA (Supplementary Figure 2). Above all, our results showed that ALKBH10B negatively affects the expression of ABA response genes.

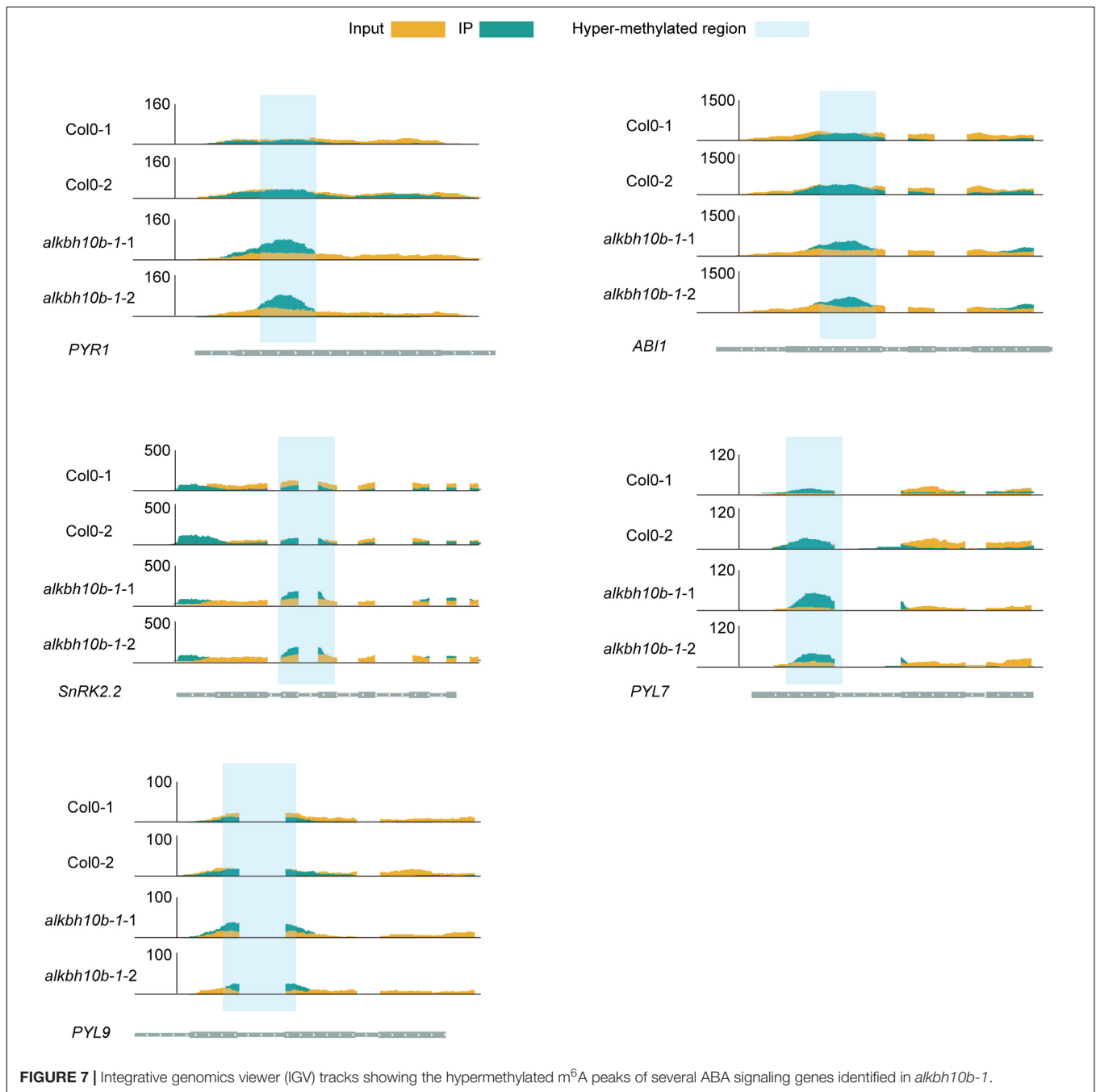
Transcriptome-Wide Analysis of mRNA m^6A Modification in *alkbh10b-1* After ABA Treatment

To investigate whether m^6A methylation pattern is different in *alkbh10b* mutant compared to wild type under 50 μM ABA treatment, we performed m^6A -seq in *alkbh10b-1* and Col-0, respectively. After removing PCR duplication, approximately 10.0–21.6 million reads were mapped to TAIR10 genome



with a more than 90% mapping rate (**Supplementary Dataset 1**). We used MACS2, a well-known peak caller, to identify peaks with a criteria of fold enrichment (IP/input) ≥ 2 and FDR ≤ 0.05 . The m^6A peaks identified in both replicates

were used for further analysis. We identified 10,138 m^6A peaks corresponding to 10,017 transcripts/genes in *alkbh10b-1* mutant (**Figure 6A**), whereas 8,978 m^6A peaks corresponding to 9,610 transcripts/genes in Col-0 (**Figure 6B**), indicating that



loss of function of ALKBH10B caused an increase of m⁶A peak numbers. We subsequently identified hypermethylated m⁶A peaks in *alkbh10b-1* mutant under ABA treatment using the MeTDiff R package software (FDR ≤ 0.05). Compared with WT, a total of 4,676 peaks were identified in *alkbh10b-1*, corresponding to 4,047 transcripts/genes (Figure 6C and Supplementary Dataset 3), which were mainly mRNA (94.17%), only ~5.13% were lncRNA and a small proportion were other RNAs (Figure 6D). Then we examined the distribution profiles of these hypermethylated m⁶A peaks within the transcripts, and found that the density of these peaks tends to be enriched

in CDS, near the start codon and 5'UTR (Figure 6E), which is consistent with the distribution of hypermethylated peaks in normal condition (Duan et al., 2017). To further locate these m⁶A peaks, we assigned these m⁶A peaks into five-non-overlapping regions and found that these hypermethylated peaks were dominantly enriched in CDS (67.58%), then 3'UTR (15.72%) and 5'UTR (15.33%) (Figure 6F). To explore the function of the hypermethylated m⁶A genes in *alkbh10b-1* mutant under ABA treatment, we performed GO enrichment analysis using DAVID. The results revealed that genes with hypermethylated m⁶A peaks are enriched in various abiotic

biological processes including response to water deprivation, cold, ABA, and heat (Figure 6G). KEGG pathway analysis showed that genes with hypermethylated m⁶A peaks are mainly enriched in biosynthesis of amino acids, pyruvate metabolism and plant hormone signal transduction (Figure 6H). In addition, several ABA signaling genes, including *PYR1*, *PYL7*, *PYL9*, *ABI1*, and *SnRK2.2* were m⁶A hypermethylated in *alkbh10b-1* under ABA treatment compared with WT (Figure 7). We also analyzed the m⁶A-seq data (Duan et al., 2017) between *alkbh10b-1* and Col-0 under normal conditions. None of these genes were m⁶A hypermethylated in *alkbh10b-1* under normal conditions (Supplementary Figure 3), suggesting m⁶A hypermethylation of these genes may specific to ABA treatment. These results indicated that ALKBH10B may regulates ABA response by affecting the m⁶A level of these ABA signaling genes.

DISCUSSION

More and more studies have reported the importance of mRNA m⁶A modification in plant development, including embryo development (Zhong et al., 2008; Shen et al., 2016; Ruzicka et al., 2017), microspore development (Zhang et al., 2019), shoot apical meristem proliferation (Shen et al., 2016), trichome morphology (Arribas-Hernandez et al., 2018; Scutenaire et al., 2018; Wei et al., 2018), floral transition (Duan et al., 2017), and fruit rippling (Zhou et al., 2019). mRNA m⁶A modification also plays roles in biotic and abiotic stress response, for example, virus infection (Martinez-Perez et al., 2017), and salt stress response (Hu et al., 2021). Plant seeds need to identify and respond to different environmental factors to decide whether to germinate or not. Even after seed germination, the seedlings will feel the changes of the surrounding environment and inhibit growth in a process called post-germination developmental arrest. Abscisic acid (ABA) is an important hormone to balance early growth or inhibition. Here, we found that ALKBH10B, an mRNA m⁶A demethylase, modulates seed germination by regulating ABA response genes.

We demonstrated that ALKBH10B plays a role in seed germination through an ABA dependent manner based on the following evidence. First, the expression of *ALKBH10B* is induced by ABA, NaCl, and mannitol stress, however, in *abi1-1*, the inducibility of *ALKBH10B* is disappeared (Figure 1 and Supplementary Figure 1), suggesting that *ALKBH10B* is regulated by ABA signaling and work downstream of ABI1. Second, the seeds of *alkbh10b* mutants were more sensitive than that of the wild type in response to ABA, osmotic, and salt stress, and showed lower germination rate and cotyledons greening at the same day (Figures 2, 3). Third, RNA-seq data reveals that, ABA response genes were upregulated in *alkbh10b-1* after ABA treatment. We found that transcription factors, *ABI3* and *ABI5*, which are two well-known positive regulators of ABA signaling during seed germination, were upregulated in *alkbh10b-1* after ABA treatment (Figures 4, 5). Fourth, some ABA response genes were m⁶A hypermethylated in *alkbh10-1*, including *PYR1*, *PYL4*, *PYL7*, *PYL9*, and *ABI1*, suggesting the role of ALKBH10B-mediated m⁶A demethylation in ABA signaling gene regulation.

Taken together, our results indicate that ALKBH10B modulated seed germination by negatively affecting ABA response genes.

The mechanism of m⁶A-mediated gene regulated is complicated. So far, researchers have found virois mechanism in mammals, including RNA stability maintenance, alternative splicing, mRNA nuclear export, translation regulation, and pri-miRNA processing (Fustin et al., 2013; Wang et al., 2014, 2015; Alarcon et al., 2015; Meyer et al., 2015; Xiao et al., 2016). Recently, some reports found that RNA m⁶A modification can modulate chromatin accessibility in mammalian cells, mainly through its “writer” or “reader” proteins to recruit histone modification proteins to change the histone modification state (Li et al., 2020; Liu et al., 2020, 2021; Xu et al., 2021), increasing the complexity of m⁶A regulation mechanism. Although, we found ALKBH10B negatively regulates the expression of ABA signaling genes under ABA treatment, and found some ABA response genes were m⁶A hypermethylated, the specific mechanism of how ALKBH10B modulates the expression of ABA response genes needs further study.

CONCLUSION

Here, we found that the expression of *ALKBH10B* is induced in response to ABA, osmotic, and salt stress, which depends on ABI1. The *alkbh10b* mutants showed more sensitive to ABA, osmotic, and salt stress during seed germination than the wild type. The expression of several ABA response genes was upregulated in *alkbh10b-1* than that of wild type, indicating ALKBH10B negatively affects the ABA signaling. Furthermore, mRNA m⁶A sequencing found that mRNA of ABA signaling genes, including *PYR1*, *ABI1*, *PYL7*, *PYL9*, and *SnRK2.2* were m⁶A hypermethylated in *alkbh10b-1* after ABA treatment. However, further studies are needed to explain the specific mechanism of how ALKBH10B modulating the expression of ABA response genes.

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: <https://bigd.big.ac.cn/gsa/s/uPCjOJ90, PRJCA005164>.

AUTHOR CONTRIBUTIONS

JT performed the experiments with the help of HD. JY analyzed the sequencing data. GJ and JT designed the experiments, interpreted the results, and wrote the manuscript. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fpls.2021.712713/full#supplementary-material>

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