Seed Science Research

cambridge.org/ssr

Mechanism of salt-inhibited early seed germination analysed by transcriptomic sequencing

Kaiwen Xia, Aili Liu, Yizhong Wang, Wannian Yang and Ye Jin

Hubei Key Laboratory of Genetic Regulation and Integrative Biology, School of Life Sciences, Central China Normal University, Wuhan, 430079, China

Research Paper

Cite this article: Xia K, Liu A, Wang Y, Yang W, Jin Y (2019). Mechanism of salt-inhibited early seed germination analysed by transcriptomic sequencing. *Seed Science Research* **29**, 73–84. https://doi.org/10.1017/S0960258519000047

Received: 2 August 2018
Revised: 7 November 2018
Accepted: 28 January 2019
First published online: 4 March 2019

Keywords:
anti-oxidant; gene expression; phytohormones; RNA-seq; salt tolerance; seed germination; seedling; transcription factor

Author for correspondence: Ye Jin, Email: jinye@mail.ccnu.edu.cn

Abstract

Seed germination, the first and critical step of the plant's life cycle, is affected by salt stress. However, the underlying mechanism of salt tolerance during early seed germination remains elusive. Here, a comparative RNA-seq analysis was performed using early germinating seeds either under normal conditions or in 100 and 150 mM sodium chloride. A total of 575 genes were up-regulated and 913 genes were down-regulated in the presence of 100 mM NaCl. Under the 150 mM NaCl treatment 1921 genes were up-regulated and 3501 genes were down-regulated. A total of 379 or 863 genes were up-regulated or down-regulated in both 100 and 150mM NaCl. These co-regulated genes were further analysed by GO enrichment. Genes in the categories *abscisic acid signaling and synthesis* and *nutrient reservoir activity* were significantly enriched in the up-regulated genes. Transcription factors responsive to gibberellin and auxin were significantly down-regulated by salinity stress. Genes related to *anti-oxidant activity* were significantly enriched in the down-regulated gene clusters by NaCl treatment. Our results suggest that salt stress inhibits seed germination by activating ABA synthesis and signalling, and depressing GA and auxin signalling, while preserving nutrition and down-regulated anti-oxidant activity. Our study provides more insight into the molecular mechanism of salt tolerance during early seed germination.

Introduction

Seed germination is the first step of the plant's life cycle and the basis for crop yield (Han and Yang, 2015). Knowledge of the mechanisms of seed germination is therefore important for both fundamental and applied issues. Under favourable conditions, seed germination begins with imbibition, followed by radicle protrusion, and elongates through the covering layers, followed by seedling development (Weitbrecht *et al.*, 2011). Seed germination is regulated by many factors, including ambient temperature, light conditions (Shi *et al.*, 2015), plant hormones (Chen *et al.*, 2008; North *et al.*, 2010; Wilson *et al.*, 2014) and salt (Xu *et al.*, 2017a).

Abscisic acid (ABA) not only endogenously synthesizes but also exogenously applied, promotes seed dormancy and inhibits seed germination (Finkelstein *et al.*, 2008). The PYR/PYL/RCAR (pyrabactin resistance/PYR-like/regulatory components of the ABA receptor) family of START domain proteins were identified as receptors for ABA (Ma *et al.*, 2009b; Park *et al.*, 2009). These proteins bind PP2Cs (type 2C protein phosphatases) including ABI1, ABI2 and PP2CA (Ma *et al.*, 2009b). The PP2Cs interact with SNF1-related kinase 2 (SnRK2) (Zhu, 2016). The SnRK2 family, which is activated by ABA, acts as a positive regulator of seed dormancy (Nakashima *et al.*, 2009). SnRK2s phosphorylate bZIP transcription factors such as ABI5 and ABFs (ABA-responsive element-binding factors) (Furihata *et al.*, 2006). The accumulation of the bZIP transcription factors then causes the inhibition of seed germination (Lopez-Molina *et al.*, 2001).

The balance between ABA and gibberellins (GAs), under the control of environmental factors, is widely accepted to be an important factor regulating seed germination (North *et al.*, 2010). During germination the GA content increases by up-regulation of GA biosynthesis and down-regulation of GA inactivation (Yamauchi *et al.*, 2004; Rieu *et al.*, 2008). In the presence of bioactive GA, the GA receptor GID1 (GIBBERELLIN INSENSITIVE DWARF 1) interacts with DELLA proteins (the GRAS family of transcription factors) promoted by GA. DELLA regulates hypocotyl growth by direct interaction with PIF (phytochrome interacting factor) (de Lucas *et al.*, 2008).

About 34 million hectares of irrigated land are salt-affected worldwide (FAO, 2011). Salinity has long been considered a major and wide-ranging factor that affects seed germination and early seedlings (Darwin, 1856). The high Na^+ concentrations not only suppress seed imbibition and embryo growth (Daszkowska-Golec, 2011; Khajeh-Hosseini *et al.*, 2003; Rajjou *et al.*, 2012; Radhakrishnan and Baek, 2017; Attia *et al.*, 2018) but also leads to

CAMBRIDGE UNIVERSITY PRESS

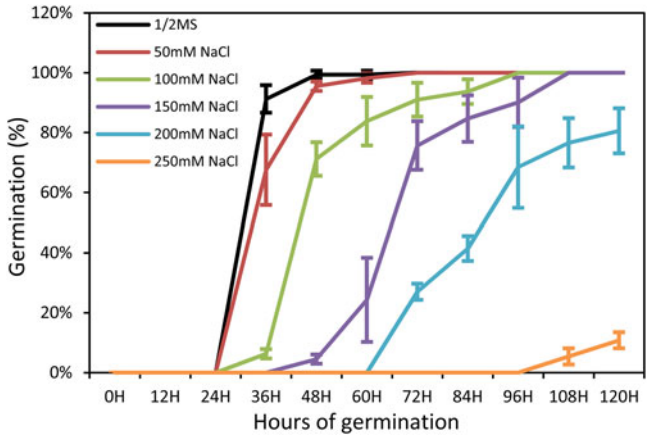

Fig. 1. Time course of seed germination in different concentrations of NaCl. Each value shown represents the mean ± SE of nine biological replicates.

inhibition of cell survival, growth and division (Zehra *et al.*, 2012). Salt tolerance in plants has been linked to a large number of factors, for example NHX1, localized to the tonoplast and SOS1 (SALT OVERLY SENSITIVE 1) localized to the plasma membrane, two key regulators to maintain low cytoplasmic Na^+ concentration in plant cells (Zhu, 2002; Deinlein *et al.*, 2014); endogenous ABA contents, which rapidly decline upon imbibition during the early phase of germination (Preston *et al.*, 2009); Ca^{2+} influx, which regulates seed germination under salt stress by modulating Na^+ accumulation through the SOS pathway (Cheng *et al.*, 2018); and the balance between ROS (reactive oxygen species) generation and ROS scavenging in plant cells (Das *et al.*, 2015).

The molecular mechanisms of salt tolerance during early seed germination remain elusive. To investigate the transcriptional regulatory network during early seed germination under salt stress, we constructed three kinds of RNA libraries with three biological replicates from Arabidopsis seeds germinated under salt stress and then performed RNA sequencing. The differentially expressed genes were analysed. These results may contribute to increase our understanding of seed early germination under salt stress.

Materials and methods

Seed germination

Seeds were surface sterilized with a 0.5% NaClO solution and then washed three times in sterilized distilled water. The sterilized seeds were imbibed in distilled water at 4°C in the dark for 48 h and then germinated on half-strength MS medium without sugar at 25°C with a 16 h photoperiod. For salt treatments, the seeds were germinated on half-strength MS with sodium chloride. For each treatment, nine replications of 40 seeds each were used. Each value is mean ± SE. Germinated seeds (2 mm radicle penetrated through seed coat) were counted every 12 h.

RNA extraction and cDNA preparation for Illumina sequencing

After treatment, the 60-h germinated seedlings were collected, immediately frozen in liquid N_2 and stored at −80°C. A total RNA kit (Invitrogen) was used to isolate total RNA from the frozen seedlings, which were then treated with RNase-free DNase (Promega) to remove genomic DNA. RNA degradation and contamination were monitored on 1% agarose gels. RNA purity was checked using the NanoPhotometer spectrophotometer (IMPLEN, CA, USA). RNA concentration was measured using a Qubit RNA Assay Kit in a Qubit 2.0 Flurometer (Life Technologies, CA, USA). RNA integrity was assessed using the RNA Nano 6000 Assay Kit of the Agilent Bioanalyzer 2100 system (Agilent Technologies, CA, USA). The cDNA for sequencing was prepared as described by Wang *et al.* (2017).

Identification of differentially expressed genes

Gene expression levels were estimated by fragments per kilobase of transcript per million fragments mapped, using the following formula:

$$\text{FPKM} = \frac{\text{cDNA Fragments}}{\text{Mapped Fragments (Millions)} \times \text{Transcript Length (kb)}}$$

Differential expression analysis of treated and untreated seedlings was performed using the DESeq R package (1.10.1). DESeq provides statistical routines for determining differential expression in digital gene expression data using a model based on a negative binomial distribution. The resulting *P* values were adjusted using Benjamini and Hochberg's approach for controlling false discovery rate. Genes with an adjusted *P*-value < 0.05 found by DESeq were assigned as differentially expressed. RT qPCR was used to confirm the expression of the differentially expressed genes (see supplemental table 3).

GO enrichment analysis

Gene ontology (GO) enrichment analysis of the differentially expressed genes (DEGs) was implemented by online AgriGO of gene ontology (http://bioinfo.cau.edu.cn/agriGO/analysis.php) as described by Wang *et al.* (2017).

Statistical analysis

Data were analysed using analysis of variance (ANOVA) followed by LSD *post-hoc* testing at $P < 0.05$. Statistical procedures were carried out with the software package SPSS statistics 17.0 as described by Xu *et al.* (2017b).

Hydrogen peroxide measurement

The seedlings germinated for 60 h were collected. The H_2O_2 concentration was measured using an Amplex red hydrogen peroxide/peroxidase assay kit (catalogue no. A22188, Thermo Fisher) as described by Wang *et al.* (2017). The 3,3′ Diaminobenzidine (DAB) staining was carried out as described by Wang *et al.* (2017).

Results

Sodium chloride inhibits seed germination

To gain more insight into the role of salt stress in seed germination, Arabidopsis (*Arabidopsis thaliana*) seeds were germinated in half-strength MS medium with different concentrations of sodium chloride. The results showed that NaCl treatment inhibited seed germination (Fig. 1). At 60 h after sowing, the

https://doi.org/10.1017/S0960258519000047 Published online by Cambridge University Press

Table 1. Summary of mRNA sequencing database

ID	Total reads	Mapped reads	Uniq mapped reads	Multiple map reads	Reads map to '+'	Reads map to '–'
c01	45,501,290	39,968,492 (87.84%)	36,750,234 (80.77%)	3,218,258 (7.07%)	19,528,804 (42.92%)	19,526,881 (42.92%)
c02	49,114,584	42,600,286 (86.74%)	39,194,862 (79.80%)	3,405,424 (6.93%)	20,910,674 (42.58%)	20,890,275 (42.53%)
c03	35,242,436	29,780,156 (84.50%)	27,151,470 (77.04%)	2,628,686 (7.46%)	14,543,295 (41.27%)	14,524,638 (41.21%)
c1001	46,077,438	40,358,804 (87.59%)	37,210,588 (80.76%)	3,148,216 (6.83%)	19,843,780 (43.07%)	19,829,875 (43.04%)
c1002	60,688,688	53,963,008 (88.92%)	49,862,419 (82.16%)	4,100,589 (6.76%)	26,667,646 (43.94%)	26,641,253 (43.90%)
c1003	50,821,048	45,242,298 (89.02%)	41,722,232 (82.10%)	3,520,066 (6.93%)	22,263,420 (43.81%)	22,238,712 (43.76%)
c1501	44,002,906	39,328,945 (89.38%)	37,051,072 (84.20%)	2,277,873 (5.18%)	19,522,724 (44.37%)	19,515,901 (44.35%)
c1502	34,683,368	31,110,994 (89.70%)	28,791,336 (83.01%)	2,319,658 (6.69%)	15,420,301 (44.46%)	15,415,174 (44.45%)
c1503	45,241,424	40,219,203 (88.90%)	37,200,176 (82.23%)	3,019,027 (6.67%)	19,934,711 (44.06%)	19,928,671 (44.05%)

Table 2. Genes differentially expressed in response to NaCl stress annotated with COG, GO, KEGG, KOG, NR and Swiss-Prot database

DEG set	Total	COG	GO	KEGG	KOG	NR	Swiss-Prot
c01_c02_c03_vs_c1001_c1002_c1003	1488	564	5	433	618	1488	1197
c01_c02_c03_vs_c1501_c1502_c1503	5422	2214	12	1721	2630	5422	4298

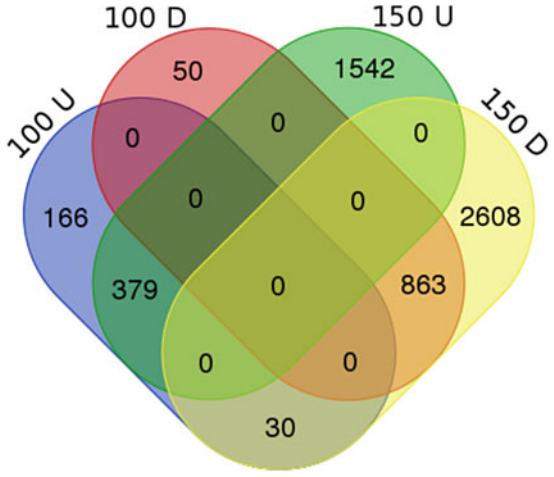

Fig. 2. Venn diagram of significantly differentially expressed genes between 100U, 100D, 150U and 150D. 100U and 150U: up-regulated genes in 100 and 150 mM NaCl, respectively; 100D and 150D: down-regulated genes in 100 and 150 mM NaCl, respectively.

germination percentage under 100 mM NaCl was 83.8%, and 24.3% under 150 mM NaCl, while the water control was 99.3% (Fig. 1). The 60-h seedlings treated with 100 and 150mM NaCl were selected for further analysis.

mRNA sequencing data mapping and annotation

Nine cDNA libraries from the control (c01, c02, c03), seedlings treated with 100 mM sodium chloride (c1001, c1002, c1003) and seedlings treated with 150 mM sodium chloride (c1501, c1502, c1503) with three biological replicates were sequenced. After removing low-quality sequences, RNA-seq produced 45501290, 49114584, 35242436, 46077438, 60688688, 50821048, 44002906, 34683368 and 45241424 clean data for c01, c02, c03, c1001, c1002, c1003, c1501, c1502 and c1503 samples, respectively. More than 84.5% of these reads can be mapped to the Arabidopsis genome. Less than 7.46% of reads had multiple alignments (Table 1). This indicated that the sequencing quality was appropriate for further analysis.

Differentially expressed genes in response to sodium chloride treatment

With a criterium of at least 2-fold difference and a p-value below 0.05 ($|\log_2 FC| \geq 1$, $p < 0.05$), 1488 genes were differentially expressed in response to 100 mM NaCl, and 5422 genes in response to 150 mM NaCl (Table 2 and supplemental table 1). The Clusters of Orthologous Groups (COG) function classification of DEGs showed that the majority of the genes were related to general function prediction only, *carbohydrate transport and metabolism*, *transcription*, *signal transduction mechanisms* and *amino acid transport and mechanism* (supplemental fig. 1). There were 913 genes down-regulated, whereas 575 genes were up-regulated in 100 mM sodium chloride. In 150 mM sodium chloride 1921 genes were up-regulated and 3501 genes were down-regulated (supplemental table 1). Of these, 379 were up-regulated and 863 genes were down-regulated in both 100 and 150 mM sodium chloride. There were 30 genes up-regulated in 100 mM sodium chloride and down-regulated in 150 mM sodium chloride. No genes were detected which were down-regulated in 100 mM NaCl and up-regulated in 150 mM NaCl (Fig. 2 and supplemental table 2).

Salt stress induces the expression of genes responsive to the nutrient reservoir

To get an overview of the physiological processes in which these commonly regulated genes were involved, GO enrichment

https://doi.org/10.1017/S0960258519000047 Published online by Cambridge University Press

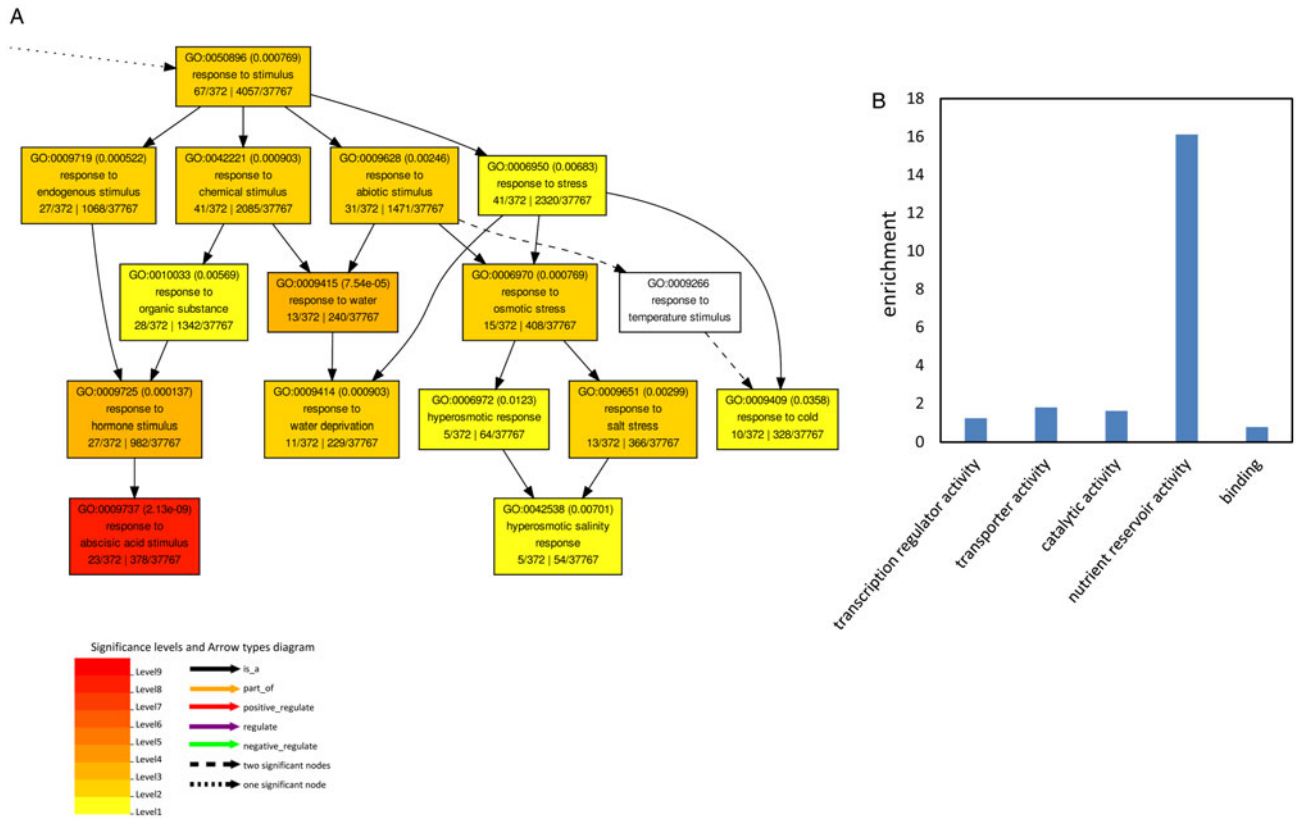

Fig. 3. Gene ontology (GO) analysis of the up-regulated genes in 100 and 150 mM NaCl. (A) Significantly enriched biological process GO terms for the co-upregulated genes. (B) Enrichment of significant molecular function of the co-upregulated genes. Enrichment was calculated by: (annotated/total number in query list)/(annotated/total number in background or reference).

Table 3. Summary of the up-regulated nutrient-reservoir related genes

	Gene expression level (normalized reads counts)									
Name	c01	c02	c03	c1001	c1002	c1003	c1501	c1502	c1503	Description
AT1G03880	0.73	0.61	0.47	3.16	1.96	3.28	26.73	14.27	20.11	CRU2 (CRUCIFERIN 2);
AT4G28520	9.19	4.90	3.52	47.07	29.33	51.18	206.07	173.94	219.79	12S seed storage protein CRU3 (CRUCIFERIN 3);
AT5G44120	28.75	8.33	12.60	148.35	70.05	123.90	872.90	753.61	650.27	12S seed storage protein CRA1 (CRUCIFERINA);
AT2G18540	0.72	0.60	1.03	7.69	18.40	10.60	74.59	101.68	78.54	Cupin family protein
AT2G28490	3.33	2.62	1.96	27.70	23.80	25.89	306.06	314.73	194.29	Cupin family protein
AT4G36700	0.75	0.52	0.52	4.21	10.21	6.39	40.43	58.50	50.92	Cupin family protein
AT4G27140	1.49	1.60	0.85	4.72	3.92	8.16	53.49	21.93	34.00	2S seed storage protein 1
AT4G27150	4.18	2.29	2.80	25.41	18.91	17.39	120.85	81.59	99.29	2S seed storage protein 2
AT3G22640	3.53	1.95	2.18	15.90	8.84	8.13	238.16	186.23	110.32	Nutrient reservoir PAP85
AT5G54740	5.15	5.70	2.53	11.57	17.53	14.84	111.27	80.47	127.85	Lipid transfer protein (LTP) family protein protease inhibitor

analysis for biological process and molecular function was performed. No significant enrichment of GO terms was observed for the commonly regulated genes up-regulated in 100 mM NaCl and down-regulated in 150 mM NaCl. Biological process GO terms related to *reproduction*, *reproductive process*, *developmental process*, *multicellular organismal*, *localization* and *response to stimulus* were significantly enriched in the up-regulated genes by NaCl treatment (Fig. 3A and supplemental fig. 2).

Molecular function GO terms related to *transcription regulator activity*, *transporter activity*, *catalytic activity*, *nutrient reservoir activity* and *binding* were significantly enriched in the up-regulated genes under NaCl treatment (Fig. 3B). Among them

https://doi.org/10.1017/S0960258519000047 Published online by Cambridge University Press

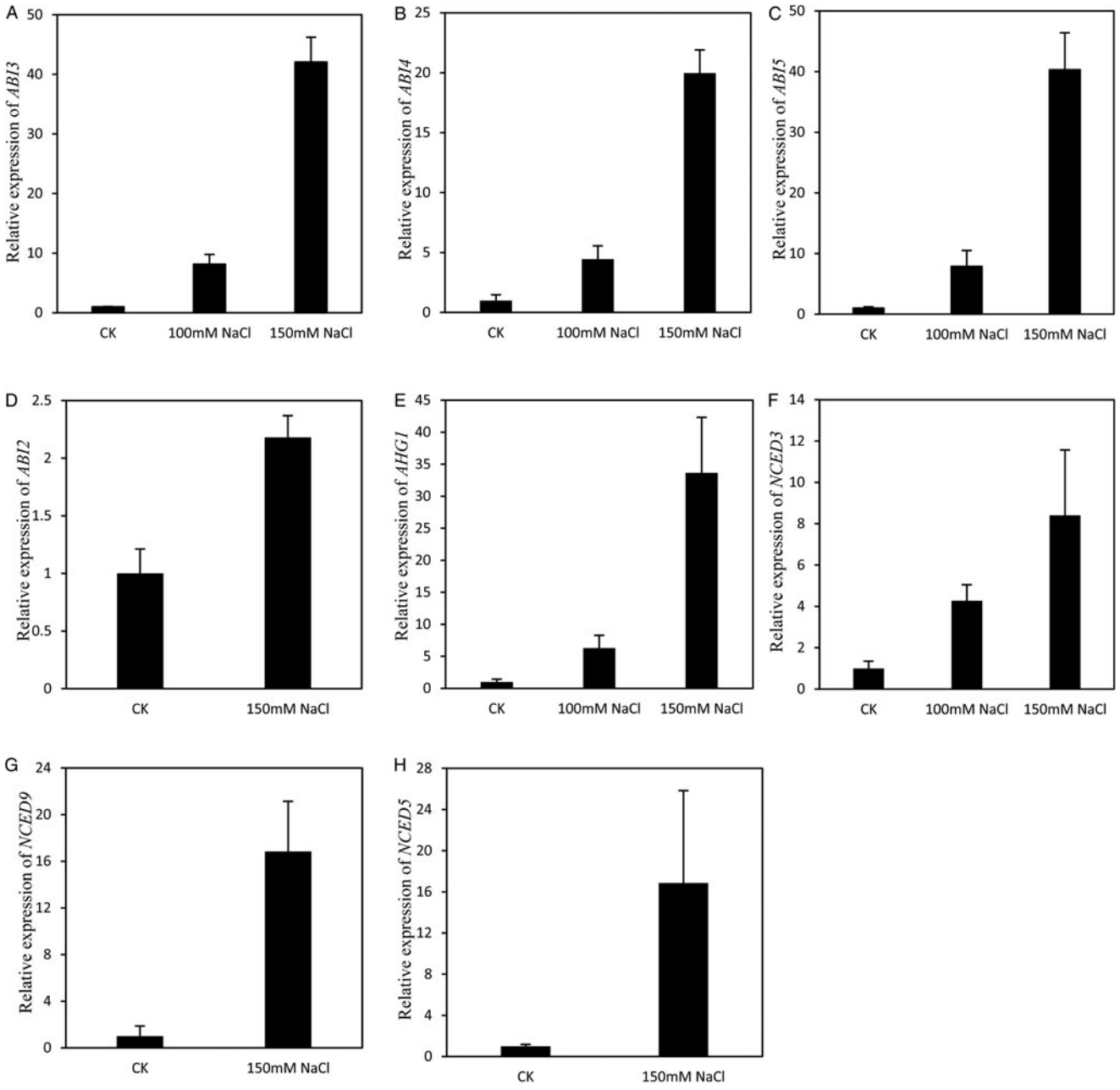

Fig. 4. Genes response to Abscisic acid stimulus were up-regulated by sodium chloride treatment. (A) Expression of ABI3 At2g40220 was up-regulated by both 100 and 150 mM NaCl treatment. (B) Expression of ABI4 At2g40220 was up-regulated by both 100 and 150 mM NaCl treatment. (C) Expression of ABI5 At2g36270 was up-regulated by both 100 and 150 mM NaCl treatment. (D) Expression of ABI2 At5g57050 was up-regulated by 150 mM NaCl treatment. (E) Expression of AHG1 At5g51760 was up-regulated by both 100 and 150 mM NaCl treatment. (F) Expression of NCED3 At3g14440 was up-regulated by both 100 and 150 mM NaCl treatment. (G) Expression of NCED9 At1g78390 was up-regulated by 150 mM NaCl treatment. (H) Expression of NCED5 At1g30100 was up-regulated by 150 mM NaCl treatment. Each value is mean ± SE of three biological replicates.

the GO terms associated with *nutrient reservoir activity* were significantly enriched in the up-regulated genes, for example the expression of AT1G03880, AT4G28520 and AT5G44120, coding for seed storage proteins of which the phosphorylation state is modulated in response to ABA (Wan *et al.*, 2007), was induced by both 100 and 150 mM NaCl (Table 3). Salinity stress also induced the expression of Cupin family proteins (AT2G18540, AT2G28490 and AT4G36700) and 2S seed storage proteins (AT4G27140 and AT4G27150) (Table 3). Taken together this suggests that under salt stress, seedlings resort to the synthesis of storage proteins compared with non-stressed conditions.

Salt stress increases the expression of ABA-responsive genes

GO terms related to *response to abscisic acid stimulus* were especially enriched in the cluster of up-regulated genes (Fig. 3A). Several ABA-insensitive (ABI) genes were obviously induced by salt stress. For example, the expression of *ABI5* was increased by almost eight times in 100 mM NaCl and more than 40 times in 150 mM NaCl (Fig. 4C). The transcriptional level of *ABI3*, a positive regulator of *ABI5*, was also induced by salt stress (Fig. 4A). In addition, salinity stress significantly increased the expression of *ABI4* (Fig. 4B). The expression of *ABI2* was induced by the 150 mM NaCl treatment (Fig. 4D). *AHG1*

https://doi.org/10.1017/S0960258519000047 Published online by Cambridge University Press

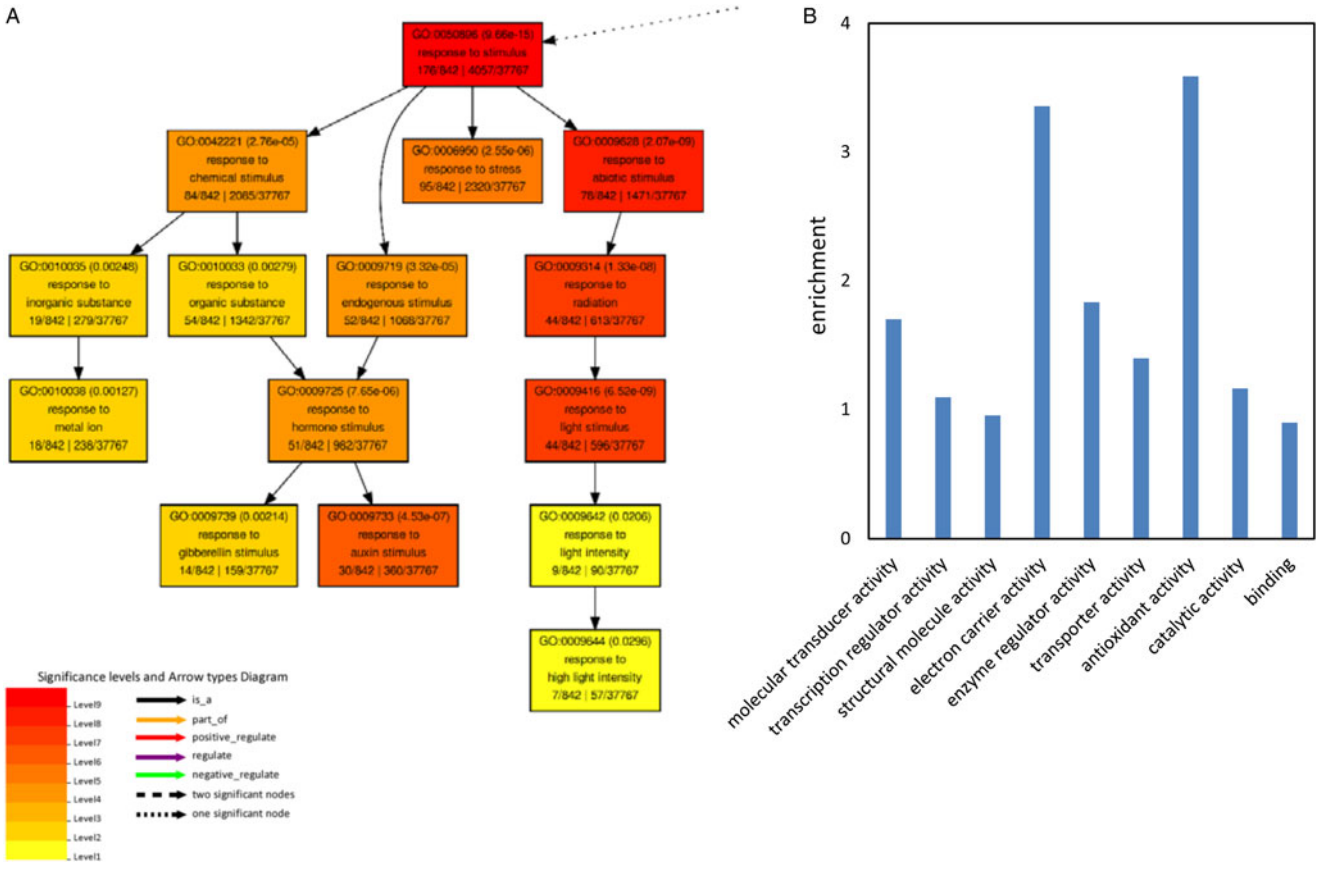

Fig. 5. GO analysis of the genes down-regulated by both 100 and 150 mM NaCl. (A) Significantly enriched biological process GO terms. (B) Enrichment of significant molecular function of the co-downregulated genes. The enrichment was calculated by: (annotated/total number in query list)/(annotated/total number in background or reference).

(ABA-hypersensitive germination 1), one of the highest expressed PP2C phosphatases of clade A in germinating seeds (Née *et al.*, 2017), was clearly induced by both 100 and 150 mM salt stress (Fig. 4E). Salt stress not only induced the expression of ABA signalling genes but also up-regulated ABA biosynthesis genes. The mRNA content of *NCED3*, coding a key enzyme 9-cis-epoxycarotenoid dioxygenase in ABA biosynthesis, increased by more than four times in 100 mM NaCl and more than eight times in 150 mM NaCl-treated samples (Fig. 4F). NaCl at 150 mM induced the expression of *NCED9* and *NCED5* (Fig. 4G,H). The up-regulation of ABA relative genes by NaCl treatment suggested that salt stress inhibited seed germination by increasing endogenous ABA synthesis and activating ABA signalling.

Transcription factors responsive to gibberellin and auxin are significantly down-regulated by salinity stress

Biological process GO terms associated with *response to stimulus* were significantly enriched in the down-regulated genes under NaCl treatment (Fig. 5A and supplemental fig. 3). GO terms related to *response to gibberellin stimulus* and *response to auxin stimulus* were especially enriched (Fig. 5A). Fourteen gibberellin related genes were significantly down-regulated by both 100 and 150 mM NaCl, of which eight encode MYB domain transcription factors (Fig. 6). The expression of *MYB28* (*AT5G61420*) was reduced to less than 0.5% by 150 mM NaCl (Fig. 6A). The expression of *MYB4* (*AT4G38620*) and *MYB60* (*AT1G08810*) were reduced to less than 1% by 150 mM NaCl treatment compared with the control (Fig. 6B,C). The mRNA content of *MYB6* (*AT4G09460*), *MYB16* (*AT5G15310*), *MYBR1* (*AT5G67300*) and another MYB family gene *AT1G74840* decreased to less than 50% by 100 mM salt stress (Fig. 6D–G). All these seven MYB family genes mentioned above are also involved in auxin signal transmission.

Besides these MYB family transcription factors, four other transcription factors involved in auxin signalling were also down-regulated by salt stress (Fig. 7). *IAA18* (*AT1G51950*) and *IAA19* (*AT3G15540*), induced by indole-3-acetic acid, were down-regulated by both 100 and 150 mM NaCl treatment (Fig. 7A,B). The expression of BTB and TAZ domain protein: *BT1* (*AT5G63160*) and *BT2* (*AT3G48360*), which are involved in auxin-activated signalling pathways, were repressed to less than 10% by both 100 and 150 mM salt stress (Fig. 7C,D). These results suggest that salt stress inhibited seed germination by repressing the expression of the transcription factors that are involved in gibberellin and/or auxin signalling pathways.

Anti-oxidant activity related genes are significantly down-regulated by salinity stress

Molecular function GO terms related to *molecular transducer activity*, *transcription regulator activity*, *structural molecule activity*, *electron carrier activity*, *enzyme regulator activity*, *transporter*

https://doi.org/10.1017/S0960258519000047 Published online by Cambridge University Press

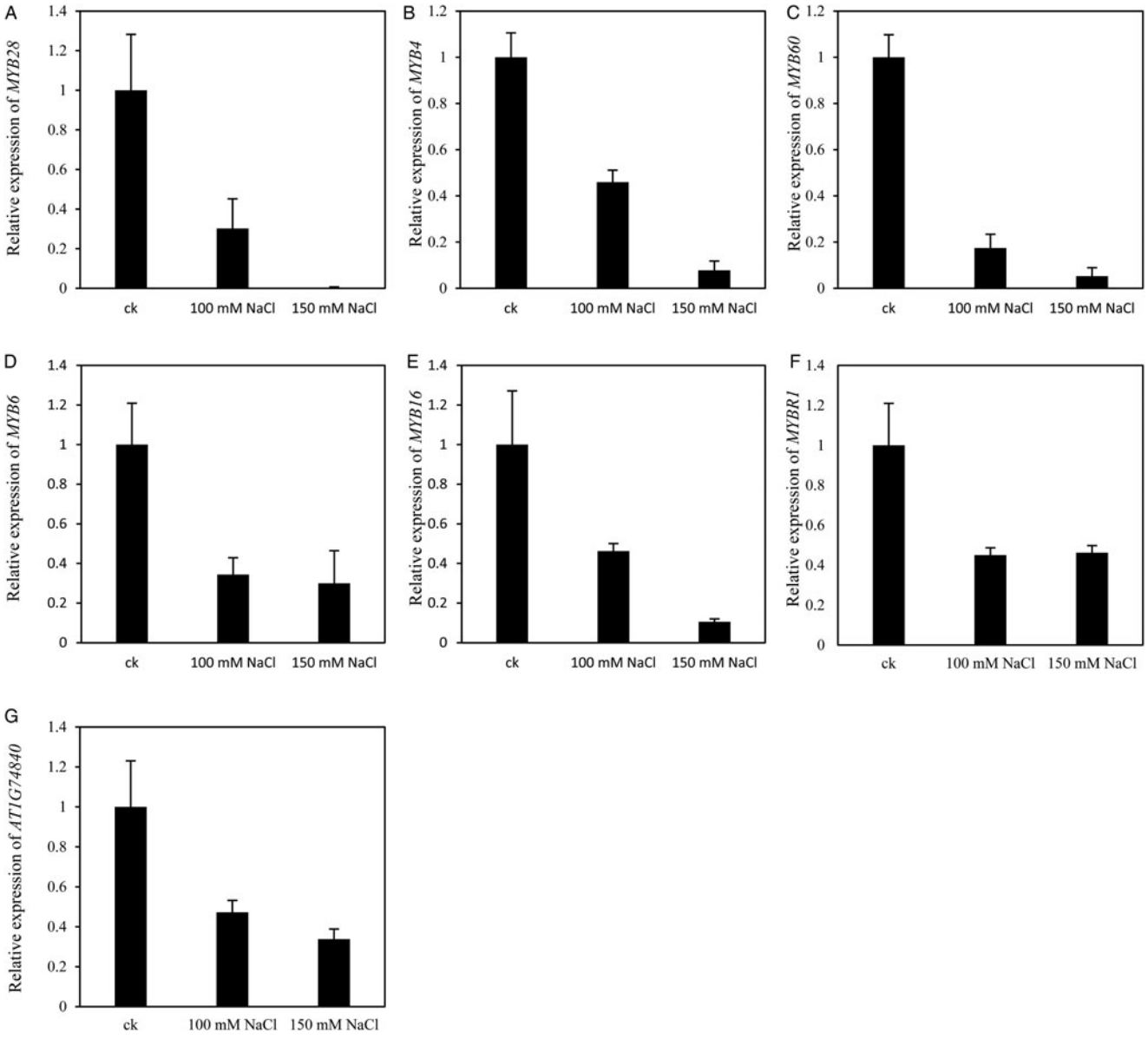

Fig. 6. MYB family transcriptional factor were down-regulated by both 100 and 150 mM sodium chloride treatment. (A) Expression of MYB28 At5g61420 was down-regulated by both 100 and 150 mM NaCl treatment. (B) Expression of MYB4 At4g38620 was down-regulated by both 100 and 150 mM NaCl treatment. (C) Expression of MYB60 At1g08810 was down-regulated by both 100 and 150 mM NaCl treatment. (D) Expression of MYB6 At4g09460 was down-regulated by both 100 and 150 mM NaCl treatment. (E) Expression of MYB16 At5g15310 was down-regulated by both 100 and 150 mM NaCl treatment. (F) Expression of MYBR1 At5g67300 was down-regulated by both 100 and 150 mM NaCl treatment. (G) Expression of At1g74840 was down-regulated by both 100 and 150mM NaCl treatment. Each value is mean ± SE of three biological replicates.

activity, *antioxidant activity*, *catalytic activity* and *binding* were significantly enriched in the down-regulated genes by NaCl treatment (Fig. 5B).

Notably genes linked with *anti-oxidant activity* were significantly enriched in down-regulated genes by NaCl treatment. Twelve genes were down-regulated in both 100 and 150 mM NaCl (Fig. 8). The expression of *AT4G25100*, coding for Fe-superoxide dismutase (FSD1), was repressed to less than 40% by both 100 and 150 mM salt stress (Fig. 8H). Another 11 genes encoding peroxidase were down-regulated by both 100 and 150 mM NaCl, among them *AT2G25080* and *AT4G31870* coding for glutathione peroxidase (Fig. 8B,I), *AT1G77490* and *AT4G09010* coding for ascorbate peroxidases (Fig. 8A,G). The expression of *AT2G38390* coding for a peroxidase could not be detected in 150 mM NaCl treated samples (Fig. 8E). Compared with the control, the expression of *AT2G38380*, *AT4G33420* and *AT5G64110* was decreased to less than 1% by treatment with 150 mM NaCl (Fig. 8D,J,L). Taken together, this suggests that salt stress represses the expression of peroxidase, thereby inhibiting seed germination. Furthermore, both DAB staining and the quantitative assay showed higher H_2O_2 levels in seedlings treated with both 100 and 150 mM NaCl than in control seedlings (Fig. 9), which further supports the notion that salt stress induced H_2O_2 accumulation.

https://doi.org/10.1017/S0960258519000047 Published online by Cambridge University Press

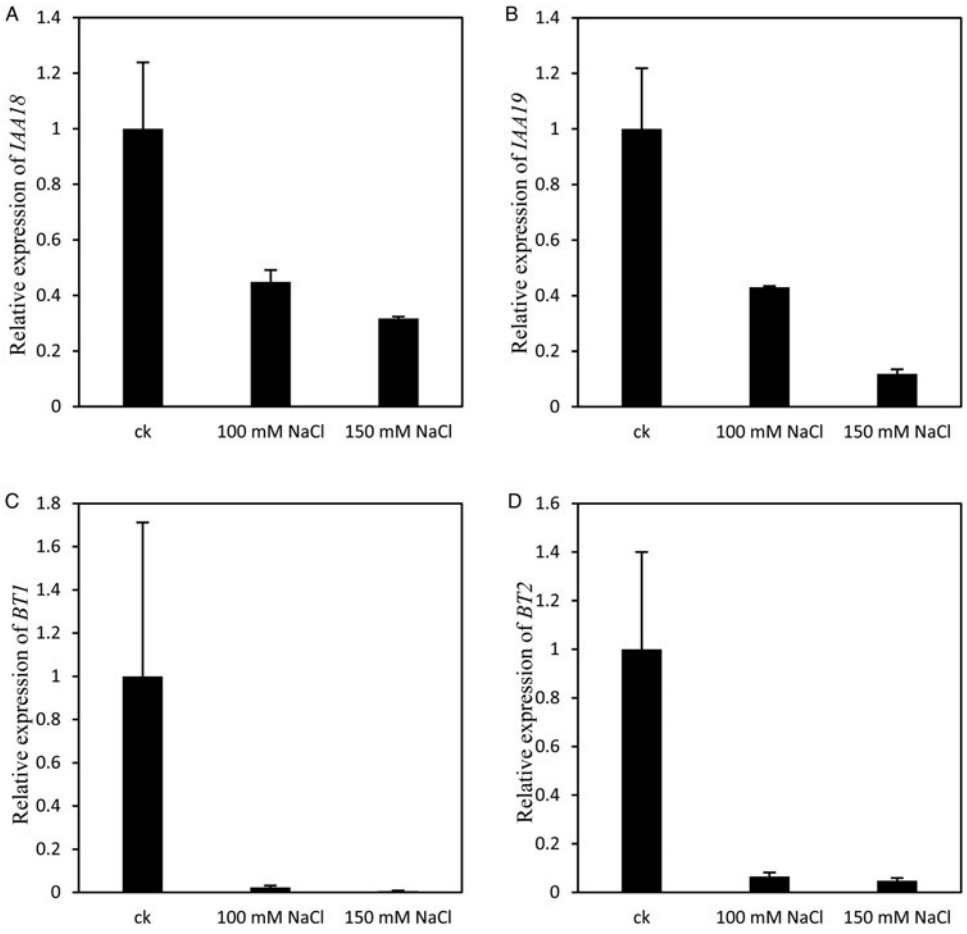

Fig. 7. transcriptional factor involved in auxin signaling were down-regulated by both 100 and 150 mM sodium chloride treatment. (A) Expression of IAA18 At1g51950 was down-regulated by both 100 and 150 mM NaCl treatment. (B) Expression of IAA19 At3g15540 was down-regulated by both 100 and 150 mM NaCl treatment. (C) Expression of BT1 At5g63160 was down-regulated by both 100 and 150 mM NaCl treatment. (D) Expression of BT2 At3g48360 was down-regulated by both 100 and 150 mM NaCl treatment. Each value is mean ± SE of three biological replicates.

Discussion

Seed germination is a key step in plant growth and development. So far, transcriptome research on salt stress regulation has focused on seedlings more than 7 days after sowing. These results showed that the salt stress responsive genes belong to the GO categories *response to stimulus*, *hormone metabolism* and *accumulation of reactive oxygen species* (Wang *et al.*, 2013; Shen *et al.*, 2014; Cheng *et al.*, 2015; Zhao *et al.*, 2017).

However, early germination is sensitive to various stresses as embryonic cells switch from quiescence to a state of high metabolic activity (Weitbrecht *et al.*, 2011). Salt stress is one of the factors affecting early seed germination (Fig. 1). Thus, investigating the molecular processes underlying early seed germination under salt stress may increase our understanding of seed germination under salt stress and help to engineer more robust species to improve yield and quality.

In the present study we identified the differentially expressed genes (DEGs) at a change of >2.0-fold and $p < 0.05$ in response to salt stress. Under 100 mM NaCl stress, 1488 genes were differentially expressed. Of these, 575 genes were up-regulated and 913 genes were down-regulated (supplemental table 1). Under 150 mM NaCl stress, 5422 genes were differentially expressed, of which 1921 genes were up-regulated and 3501 genes were down-regulated (supplemental table 2). As 65.9% up-regulated genes and 94.5% down-regulated genes under 100 mM NaCl stress were also up- or down-regulated under 150 mM NaCl stress (Fig. 2), we further analysed the collective up- and down-regulated genes. Biological process GO terms related to *response to stimulus* were significantly enriched in both.

High salinity inhibits germination by regulating ABA biosynthesis and signalling

Seed germination is usually accompanied by the increases in ABA catabolism (Vishwakarma *et al.*, 2017), ABA signalling protein SNF1-RELATED PROTEIN KINASE2.2 (SnRK2.2) and SnRK2.3 inhibitor seed germination (Fujii *et al.*, 2007). ABI1 and ABI2, type 2C protein phosphatases that interact with SnRK2 family, perform negative regulation of germination of seeds (Finkelstein and Somerville, 1990). ABI4, a key factor that regulates primary seed dormancy, mediates the balance between ABA and GA biosynthesis (Shu *et al.*, 2013). The accumulation of ABI5, a bZIP transcription factor, causes the inhibition of seed germination (Lopez-Molina *et al.*, 2001). In our study, GO terms related to *response to abscisic acid stimulus* were especially enriched in the

https://doi.org/10.1017/S0960258519000047 Published online by Cambridge University Press

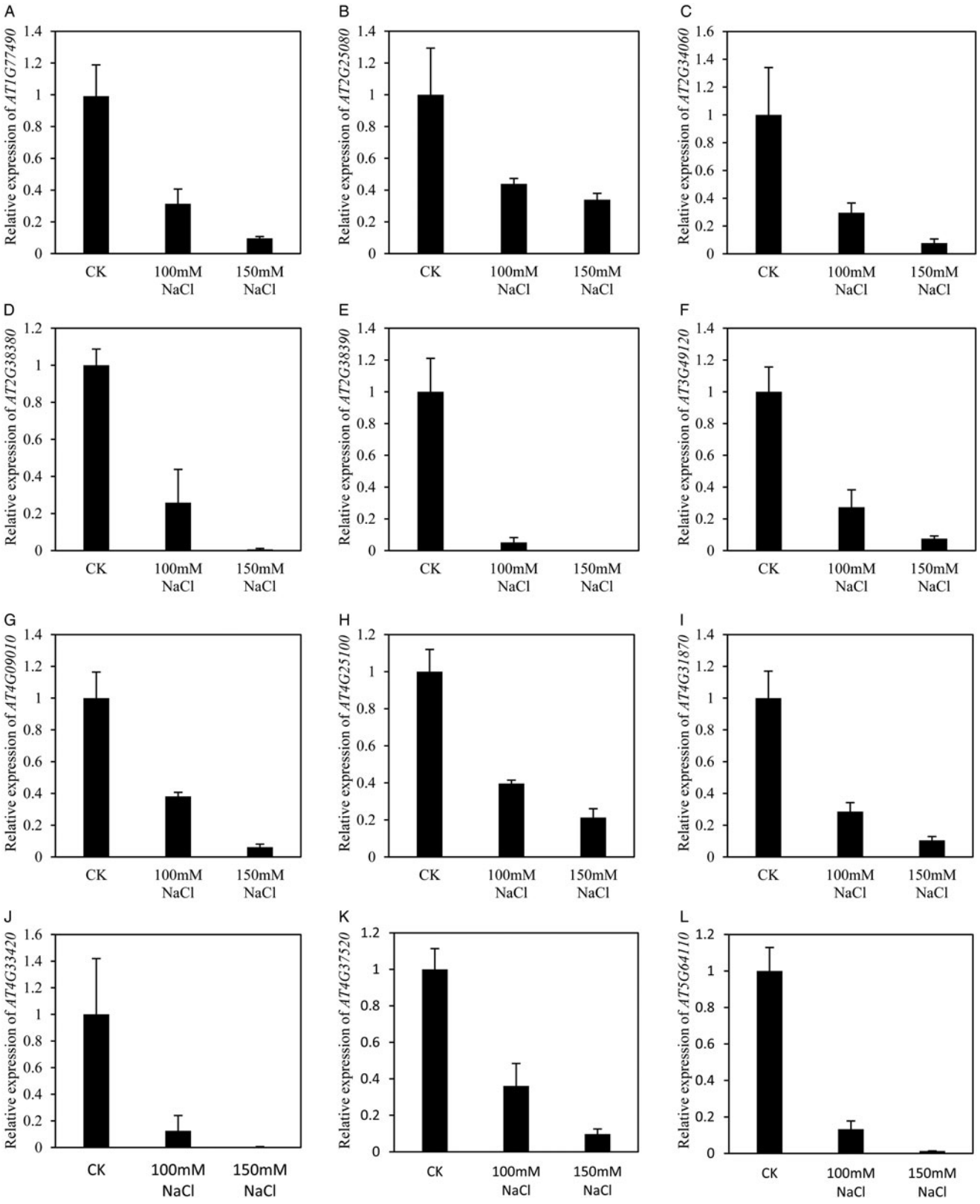

Fig. 8. Genes response to antioxidant activity were down-regulated by both 100 and 150 mM sodium chloride treatment. (A) Expression of At1g77490 was down-regulated by both 100 and 150 mM NaCl treatment. (B) Expression of At2g25080 was down-regulated by both 100 and 150 mM NaCl treatment. (C) Expression of At2g34060 was down-regulated by both 100 and 150 mM NaCl treatment. (D) Expression of At2g38380 was down-regulated by both 100 and 150 mM NaCl treatment. (E) Expression of At2g38390 was down-regulated by both 100 and 150 mM NaCl treatment. (F) Expression of At3g49120 was down-regulated by both 100 and 150 mM NaCl treatment. (G) Expression of At4g09010 was down-regulated by both 100 and 150 mM NaCl treatment. (H) Expression of At4g25100 was down-regulated by both 100 and 150 mM NaCl treatment. (I) Expression of At4g31870 was down-regulated by both 100 and 150 mM NaCl treatment. (J) Expression of At4g33420 was down-regulated by both 100 and 150 mM NaCl treatment. (K) Expression of At4g37520 was down-regulated by both 100 and 150 mM NaCl treatment. (L) Expression of At5g64110 was down-regulated by both 100 and 150 mM NaCl treatment. Each value is mean ± SE of three biological replicates.

https://doi.org/10.1017/S0960258519000047 Published online by Cambridge University Press

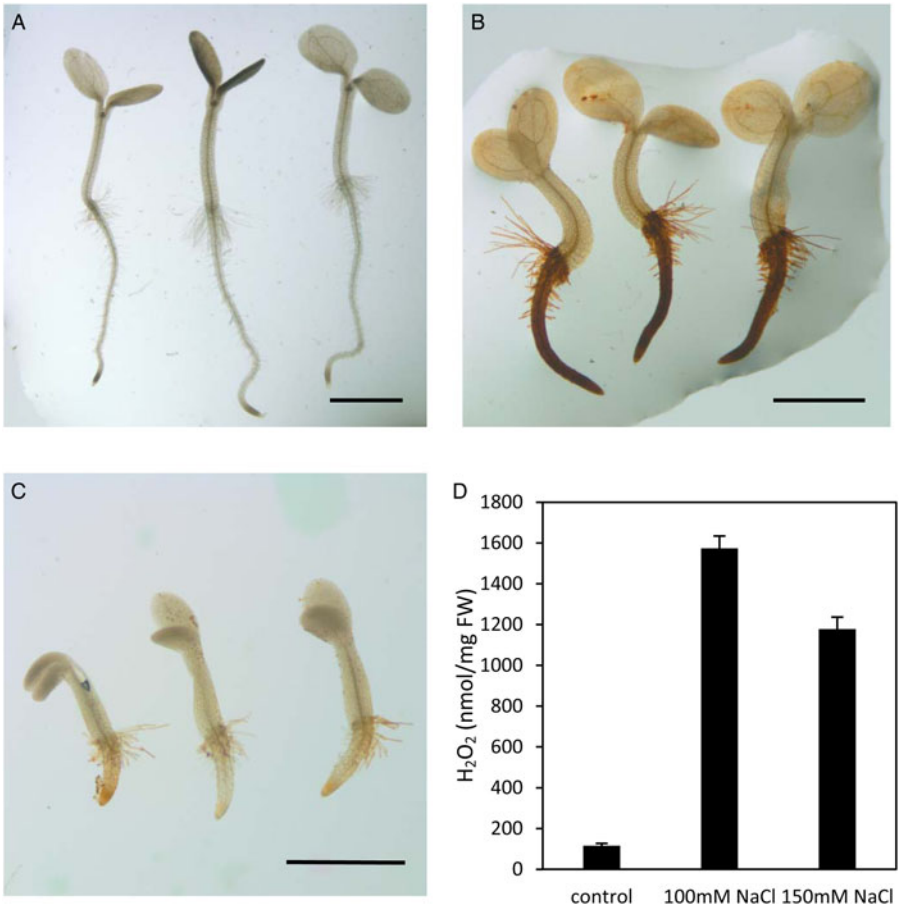

Fig. 9. Accumulated H_2O_2 content as detected by DAB staining in 60-hour-old seedlings under salt stress. (A) Water control; (B) 100 mM NaCl; (C) 150 mM NaCl; (D) H_2O_2 content quantified with or without NaCl. Each value represents the mean ± SE of six biological replicates. Scale bar, 1 mm.

up-regulated gene set upon treatment by 100 and 150 mM NaCl (Fig. 3). The expression of *ABI3*, *ABI4* and *ABI5* was significantly up-regulated by both 100 and 150 mM NaCl treatment, whereas the expression of *ABI2* was induced by 150 mM NaCl treatment (Fig. 4). NCED genes encode 9-cis-epoxycarotenoid dioxygenases, which catalyse the first step of ABA biosynthesis. The expression of *NCED1* decreased rapidly during seed germination in maize (Li *et al.*, 2017). Here the expression of *NCED3* was notably up-regulated by both 100 and 150 mM salt stress and the expression of *NCED5* and *NCED9* was increased by 150 mM NaCl (Fig. 4). These results suggest that salt stress inhibits seed germination by enhancing ABA biosynthesis and signalling.

Salt stress represses the expression of transcription factors involved in phytohormone signalling

Salt stress not only repressed the expression of transcription factors related to ABA signalling but also induced the expression of transcription factors associated with GA and auxin signalling (Figs 6 and 7). The role of transcription factors in salt stress has been demonstrated in a range of plant species. For instance, over-expression of a NAC transcription factor gene, cloned from *Thellungiella halophile*, especially improved salt stress tolerance in Arabidopsis by a positive regulation of ion transport (Liu *et al.*, 2018). The MYB family, characterized by the N-terminus conserved MYB repeats, are involved in the regulation of biotic and abiotic stresses (Roy, 2016). In Arabidopsis, MYB4 controls production of UV-protecting sunscreens (Jin *et al.*, 2000). MYB60 and MYB96 regulate many plant responses to drought stress through ABA signalling (Seo and Park, 2010). The MYB genes *OsMYB2*, *OsMYB3R-2* and *OsMYB4* from rice have been identified as important components of plant abiotic stress response pathways (Vannini *et al.*, 2004; Ma *et al.*, 2009a; Yang *et al.*, 2012). In this context, our study shows that the MYB family of transcription factors is depressed by salt stress (Fig. 6). Besides the MYB transcription factors, the transcription factors *IAA18*, *IAA19*, *BT1* and *BT2* were also down-regulated by salt stress (Fig. 7). These results suggest that transcription factors involved in phytohormone signalling are important factors of the salt stress response pathway in Arabidopsis.

Salt stress represses the expression of genes related to anti-oxidant response

Reactive oxygen species (ROS), including the superoxide hydrogen peroxide and hydroxyl free radicals, are assumed to be linked to seed germination (Apel and Hirt, 2004). During imbibition the fast water uptake and activation of respiration may result in high ROS generation, which can lead to DNA oxidative damage (Macovei *et al.*, 2017). In addition, ROS are also involved in seed germination as signalling molecules, mediated by phytohormones such as ABA, GA, ethylene, auxin, cytokinin, brassinosteroids, jasmonic acid or salicylic acid (Zhang *et al.*, 2014; Bi *et al.*, 2017; Li *et al.*, 2017; Ishibashi *et al.*, 2017; Macovei *et al.*, 2017). For example, inhibition of catalase activity and H_2O_2 generation inhibits seed germination of Col-0, *abi5* mutants and *ABI5* over-

https://doi.org/10.1017/S0960258519000047 Published online by Cambridge University Press

expression transgenic lines in Arabidopsis (Bi *et al.*, 2017). In barley, the balance between ABA and ROS regulates seed dormancy and germination (Ishibashi *et al.*, 2017). Exogenously applied ABA or GA resulted in modulating ROS levels in Arabidopsis (Ye *et al.*, 2012; Lariguet *et al.*, 2013). The ROS balance is maintained by anti-oxidant systems. During seed germination the activities of catalase (CAT), superoxide dismutase (SOD), ascorbate peroxidase (APX) and gluthatione reductase (GR) may be regulated to modulate ROS levels (Oracz *et al.*, 2009; Ye *et al.*, 2012). Here we show that molecular function GO terms such as *anti-oxidant activity* were particularly enriched in the down-regulated gene set upon treatment by 100 and 150 mM NaCl (Fig. 8). Twelve genes were down-regulated by both 100 and 150 mM NaCl (Fig. 6). Among these two genes code for APX, two for glutathione peroxidase and one for SOD. These results show that salt stress represses the expression of genes coding for peroxidases. The down-regulation of these genes may result in accumulation of ROS, which inhibit seed germination both as signaling and damaging molecules.

Author ORCIDs. Ye Jin, 0000-0002-5946-3267

Acknowledgements. This work was financially supported by the National Natural Science Foundation of China (no. 31200192), the self-determined research funds of CCNU from the colleges' basic research and operation of MOE (nos CCNU14KFY003, CNU16A05010 and CCNU18KYZHSY11), and a project of the Hubei Key Laboratory of Genetic Regulation and Integrative Biology (no. GRIB201705).

Author contributions. Y.J. designed research. A.L., K.X. and Y.J. carried out most of the experiments. A.L., K.X., Y.W., W.Y. and Y.J. analysed data. Y.J. wrote the paper. Y.W. and W.Y. revised the manuscript. All authors read and approved the final manuscript.

Data availability. The datasets analysed for this study can be found in the SRA database: https://www.ncbi.nlm.nih.gov/sra/SRP150903

Supplementary material. To view supplementary material for this article, please visit https://doi.org/10.1017/S0960258519000047.

Supplemental figure 1. The COG (Cluster of Orthologous Groups of proteins) function classification of differentially expressed genes (DGEs) in response to NaCl stress. (A) The COG function classification of DGEs in response to 100 mM NaCl stress. (B) The COG function classification of DGEs in response to 150 mM NaCl stress.

Supplemental figure 2. Significantly enriched biological process Gene Ontology terms for the co-upregulated genes.

Supplemental figure 3. Significantly enriched biological process Gene Ontology terms for the co-downregulated genes.

Supplemental table 1. Differentially expressed genes (DEGs) in response to 100 and 150 mM NaCl stress.

Supplemental table 2. The commonly up- and down-regulated genes in 100 and 150 mM NaCl treated samples.

Supplemental table 3. The relative expression of the genes mentioned in our manuscript, which are regulated by 100 and 150 mM NaCl, was confirmed by RT-PCR. The '±' indicates SD of four biological replicates.

References

Apel K and Hirt H (2004) Reactive oxygen species: metabolism, oxidative stress, and signal transduction. *Annual Review of Plant Biology* **55**, 373–399.

Attia H, Alamer KH, Selmi I, Djebali W, Chaïbi W and Nasri MB (2018) Physiological and structural modifications in snail medic (*Medicago scutellata* L.) plants exposed to salinity. *Acta Biologia Hungaria* **69**, 336–349.

Bi C, Ma Y, Wu Z, Yu YT, Liang S, Lu K and Wang XF (2017) Arabidopsis ABI5 plays a role in regulating ROS homeostasis by activating CATALASE 1 transcription in seed germination. *Plant Molecular Biology* **94**, 197–213.

Chen H, Zhang J, Neff MM, Hong SW, Zhang H, Deng XW and Xiong L (2008) Integration of light and abscisic acid signaling during seed germination and early seedling development. *Proceedings of the National Academy of Sciences of the USA* **105**, 4495–5000.

Cheng MC, Ko K, Chang WL, Kuo WC, Chen GH and Lin TP (2015) Increased glutathione contributes to stress tolerance and global translational changes in Arabidopsis. *Plant Journal* **83**, 926–939.

Cheng Y, Zhang X, Sun T, Tian Q and Zhang WH (2018) Glutamate receptor homolog3.4 is involved in regulation of seed germination under salt stress in Arabidopsis. *Plant and Cell Physiology* **59**, 978–988.

Darwin C (1856) On the action of sea-water on the germination of seeds. *Journal of the Proceedings of the Linnean Society London* (*Botany*) **1**, 130–140.

Das P, Nutan KK, Singla-Pareek SL and Pareek A (2015) Understanding salinity responses and adopting 'omics-based' approaches to generate salinity tolerant cultivars of rice. *Frontiers in Environmental Science* **6**, 712.

Daszkowska-Golec A (2011) Arabidopsis seed germination under abiotic stress as a concert of action of phytohormones. *OMICS* **15**, 763–764.

de Lucas M, Davière JM, Rodríguez-Falcón M, Pontin M, Iglesias-Pedraz JM, Lorrain S, Fankhauser C, Blázquez MA, Titarenko E and Prat S (2008) A molecular framework for light and gibberellin control of cell elongation. *Nature* **451**, 480–484.

Deinlein U, Stephan AB, Horie T, Luo W, Xu G and Schroeder JI (2014) Plant salt-tolerance mechanisms. *Trends in Plant Science* **19**, 371–379.

FAO (2011). The state of the world's land and water resources for food and agriculture (SOLAW) – managing systems at risk. Food and Agriculture, Organization of the United Nations, Rome and Earthscan, London.

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

Finkelstein RR and Somerville CR (1990) Three classes of abscisic acid (ABA)-insensitive mutations of Arabidopsis define genes that control overlapping subsets of ABA responses. *Plant Physiology* **94**, 1172–1179.

Fujii H, Verslues PE and Zhu JK (2007) Identification of two protein kinases required for abscisic acid regulation of seed germination, root growth, and gene expression in Arabidopsis. *Plant Cell* **19**, 485–494.

Furihata T, Maruyama K, Fujita Y, Umezawa T, Yoshida R, Shinozaki K and Yamaguchi-Shinozaki K (2006) Abscisic acid-dependent multisite phosphorylation regulates the activity of a transcription activator AREB1. *Proceedings of the National Academy of Sciences of the USA* **103**, 198–193.

Han C and Yang PF (2015) Studies on the molecular mechanisms of seed germination. *Proteomics* **15**, 1671–1679.

Ishibashi Y, Aoki N, Kasa S, Sakamoto M, Kai K, Tomokiyo R, Watabe G, Yuasa T and Iwaya-Inoue M (2017) The interrelationship between abscisic acid and reactive oxygen species plays a key role in barley seed dormancy and germination. *Frontiers in Plant Science* **8**, 275.

Jin H, Cominelli E, Bailey P, Parr A, Mehrtens F, Jones J, Tonelli C, Weisshaar B and Martin C (2000) Transcriptional repression by AtMYB4 controls production of UV- protecting sunscreens in Arabidopsis. *EMBO Journal* **19**, 6150–6161.

Khajeh-Hosseini M, Powell AA and Bingham IJ (2003) The interaction between salinity stress and seed vigour during germination of soybean seeds. *Seed Science and Technology* **31**, 715–725.

Lariguet P, Ranocha P, De Meyer M, Barbier O, Penel C and Dunand C (2013) Identification of a hydrogen peroxide signalling pathway in the control of light-dependent germination in Arabidopsis. *Planta* **238**, 381–395.

Li Z, Xu J, Gao Y, Wang C, Guo G, Luo Y, Huang Y, Hu W, Sheteiwy MS, Guan Y and Hu J (2017) The synergistic priming effect of exogenous salicylic acid and H_2O_2 on chilling tolerance enhancement during maize (*Zea mays* L.) seed germination. *Frontiers in Plant Science* **8**, 1153.

Liu C, Wang B, Li Z, Peng Z and Zhang J (2018) TsNAC1 is a key transcription factor in abiotic stress resistance and growth. *Plant Physiology* **176**, 742–756.

Lopez-Molina L, Mongrand S and Chua NH (2001) A postgermination developmental arrest checkpoint is mediated by abscisic acid and requires the ABI5 transcription factor in Arabidopsis. *Proceedings of the National Academy of Sciences of the USA* **98**, 4782–4787.

Ma QB, Dai XY, Xu YY, Guo J, Liu YJ, Chen N, Xiao J, Zhang D, Xu Z, Zhang X and Chong K (2009a) Enhanced tolerance to chilling stress in

OsMYB3R-2 transgenic rice is mediated by alteration in cell cycle and ectopic expression of stress genes. *Plant Physiology* **150**, 244–256.

Ma Y, Szostkiewicz I, Korte A, Moes D, Yang Y, Christmann A and Gril E (2009b) Regulators of PP2C phosphatase activity function as Abscisic acid sensors. *Science* **324**, 1064–1068.

Macovei A, Pagano A, Leonetti P, Carbonera D, Balestrazzi A and Araújo SS (2017) Systems biology and genome-wide approaches to unveil the molecular players involved in the pre-germinative metabolism: implications on seed technology traits. *Plant Cell Reports* **36**, 669–688.

Nakashima K, Fujita Y, Kanamori N, Katagiri T, Umezawa T, Kidokoro S, Maruyama K, Yoshida T, Ishiyama K, Kobayashi M, Shinozaki K and Yamaguchi-Shinozaki K (2009) Three Arabidopsis SnRK2 protein kinases, SRK2D/SnRK2.2, SRK2E/SnRK2.6/OST1 and SRK2I/SnRK2.3, involved in ABA signaling are essential for the control of seed development and dormancy. *Plant and Cell Physiology* **50**, 1345–1363.

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

North H, Baud S, Debeaujon I, Dubos C, Dubreucq B, Grappin P, Jullien M, Lepiniec L, Marion-Poll A, Miquel M, Rajjou L, Routaboul JM and Caboche M (2010) Arabidopsis seed secrets unravelled after a decade of genetic and omics-driven research. *Plant Journal* **61**, 971–981.

Oracz K, El-Maarouf-Bouteau H, Kranner I, Bogatek R, Corbineau F, Bailly C (2009) The mechanisms involved in seed dormancy alleviation by hydrogen cyanide unravel the role of reactive oxygen species as key factors of cellular signaling during germination. *Plant Physiology* **150**, 494–505.

Park SY, Fung P, Nishimura N, Jensen DR, Fujii H, Zhao Y, Lumba S, Santiago J, Rodrigues A, Chow TF, Alfred SE, Bonetta D, Finkelstein R, Provart NJ, Desveaux D, Rodriguez PL, McCourt P, Zhu JK, Schroeder JI, Volkman BF and Cutler SR (2009) Abscisic acid inhibits type 2C protein phosphatases via the PYR/PYL family of START proteins. *Science* **324**, 1068–1071.

Preston J, Tatematsu K, Kanno Y, Hobo T, Kimura M, Jikumaru Y, Yano R, Kamiya Y and Nambara E (2009) Temporal expression patterns of hormone metabolism genes during imbibition of Arabidopsis thaliana seeds: a comparative study on dormant and non-dormant accessions. *Plant and Cell Physiology* **50**, 1786–1800.

Radhakrishnan R and Baek KH (2017) Physiological and biochemical perspectives of non-salt tolerant plants during bacterial interaction against soil salinity. *Plant Physiology and Biochemistry* **116**, 116–126.

Rajjou L, Duval M, Gallardo K, Catusse J, Bally J, Job C and Job D (2012) Seed germination and vigor. *Annual Review of Plant Biology* **63**, 507–533.

Rieu I, Eriksson S, Powers SJ, Gong F, Griffiths J, Woolley L, Benlloch R, Nilsson O, Thomas SG, Hedden P and Phillips AL (2008) Genetic analysis reveals that C19-GA 2-oxidation is a major gibberellin inactivation pathway in Arabidopsis. *Plant Cell* **20**, 2420–2436.

Roy S (2016). Function of MYB domain transcription factors in abiotic stress and epigenetic control of stress response in plant genome. *Plant Signaling and Behavior* **11**, e1117723.

Seo PJ and Park CM (2010) MYB96-mediated abscisic acid signals induce pathogen resistance response by promoting salicylic acid biosynthesis in Arabidopsis. *New Phytologist* **186**, 471–483.

Shen X, Wang Z, Song X, Xu J, Jiang C, Zhao Y, Ma C and Zhang H (2014) Transcriptomic profiling revealed an important role of cell wall remodeling and ethylene signaling pathway during salt acclimation in Arabidopsis. *Plant Molecular Biology* **86**, 303–317.

Shi H, Wang X, Mo X, Tang C, Zhong S and Deng XW (2015) Arabidopsis DET1 degrades HFR1 but stabilizes PIF1 to precisely regulate seed germination. *Proceedings of the National Academy of Sciences of the USA* **112**, 3817–3822.

Shu K, Zhang H, Wang S, Chen M, Wu Y, Tang S, Liu C, Feng Y, Cao X and Xie Q (2013) ABI4 regulates primary seed dormancy by regulating the biogenesis of abscisic acid and gibberellins in Arabidopsis. *PLoS Genetics* **9**, e1003577.

Vannini C, Locatelli F, Bracale M, Magnani E, Marsoni M, Osnato M, Mattana M, Baldoni E and Coraggio I (2004) Overexpression of the rice OsMYB4 gene increases chilling and freezing tolerance of Arabidopsis thaliana plants. *Plant Journal* **37**, 115–127.

Vishwakarma K, Upadhyay N, Kumar N, Yadav G, Singh J, Mishra RK, Kumar V, Verma R, Upadhyay RG, Pandey M and Sharma S (2017) Abscisic acid signaling and abiotic stress tolerance in plants: a review on current knowledge and future prospects. *Frontiers in Plant Science* **8**, 161.

Wan L, Ross AR, Yang J, Hegedus DD and Kermode AR (2007) Phosphorylation of the 12 S globulin cruciferin in wild-type and abi1-1 mutant Arabidopsis thaliana (thale cress) seeds. *Biochemical Journal* **404**, 247–256.

Wang Y, Hu Q, Wu Z, Wang H, Han S, Jin Y, Zhou J, Zhang Z, Jiang J, Shen Y, Shi H and Yang W (2017) HISTONE DEACETYLASE 6 represses pathogen defence responses in *Arabidopsis thaliana*. *Plant, Cell and Environment* **40**, 2972–2986.

Wang Y, Yang L, Zheng Z, Grumet R, Loescher W, Zhu JK, Yang P, Hu Y and Chan Z (2013). Transcriptomic and physiological variations of three Arabidopsis ecotypes in response to salt stress. *PLoS ONE* **8**, e69036.

Weitbrecht K, Müller K and Leubner-Metzger G (2011) First off the mark: early seed germination. *Journal of Experimental Botany* **62**, 3289–3309.

Wilson RL, Kim H, Bakshi A and Binder BM (2014) The ethylene receptors ETHYLENE RESPONSE1 and ETHYLENE RESPONSE2 have contrasting roles in seed germination of Arabidopsis during salt stress. *Plant Physiology* **165**, 1353–1366.

Xu E, Chen M, He H, Zhan C, Cheng Y, Zhang H and Wang Z (2017a) Proteomic analysis reveals proteins involved in seed imbibition under salt stress in rice. *Frontiers in Plant Science* **7**, 2006.

Xu XX, Hu Q, Yang WN and Jin Y (2017b) The roles of cell wall invertase inhibitor in regulating chilling tolerance in tomato. *BMC Plant Biology* **17**, 195.

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

Yang A, Xiaoyan DX and Zhang WH (2012) A R2R3-type MYB gene, OsMYB2, is involved in salt, cold, and dehydration tolerance in rice. *Journal of Experimental Botany* **63**, 2541–2556.

Ye N, Zhu G, Liu Y, Zhang A, Li Y, Liu R, Shi L, Jia L and Zhang J (2012) Ascorbic acid and reactive oxygen species are involved in the inhibition of seed germination by abscisic acid in rice seeds. *Journal of Experimental Botany* **63**, 1809–1822.

Zehra A, Gul B, Ansari R and Khan MA (2012) Role of calcium in alleviating effect of salinity on germination of *Phragmites karka* seeds. *South African Journal of Botany* **78**, 122–128.

Zhang HJ, Zhang N, Yang RC, Wang L, Sun QQ, Li DB, Cao YY, Weeda S, Zhao B, Ren S and Guo YD (2014) Melatonin promotes seed germination under high salinity by regulating antioxidant systems, ABA and GA_4 interaction in cucumber (*Cucumis sativus* L.). *Journal of Pineal Research* **57**, 269–279.

Zhao P, Wang L, Zhao X, Chen G and Ma XF (2017) A comparative transcriptomic analysis reveals the core genetic components of salt and osmotic stress responses in *Braya humilis*. *PLoS One* **12**, e0183778.

Zhu JK (2002) Salt and drought stress signal transduction in plants. *Annual Review of Plant Biology* **53**, 247–273.

Zhu JK (2016). Abiotic stress signaling and responses in plants. *Cell* **167**, 313–324.