Planta (2020) 252:95
https://doi.org/10.1007/s00425-020-03502-2

ORIGINAL ARTICLE

NAC103, a NAC family transcription factor, regulates ABA response during seed germination and seedling growth in Arabidopsis

Ling Sun[1] · Li-Ping Liu[1] · Ya-Zhen Wang[1] · Lei Yang[1] · Mei-Jing Wang[2] · Jian-Xiang Liu[2]

Received: 26 September 2020 / Accepted: 21 October 2020 / Published online: 31 October 2020

Abstract

Main conclusion **The Arabidopsis transcription factor *NAC103* is up-regulated and its encoding protein is stabilized by ABA treatment, which positively regulates several ABA-responsive downstream genes during seed germination and seedlings growth.**

Abstract The Arabidopsis transcription factor NAC103 was previously found to be involved in endoplasmic reticulum (ER) stress and DNA damage responses. In this study, we report the new biological function of NAC103 in abscisic acid (ABA) response during seed germination and seedling growth in Arabidopsis. The expression of *NAC103* was up-regulated and the NAC103 protein was stabilized by ABA treatment. Both the loss-of-function mutants of *NAC103*, created by targeted gene-editing, and the over-expression plants of *NAC103* have no obvious germination-related phenotype under normal growth conditions. However, under exogenous ABA treatment conditions, the *NAC103* mutants were less sensitive to ABA during seed germination; in contrast, the *NAC103* over-expression plants were more sensitive to ABA during seed germination and young seedling growth. Further, NAC103 regulated several ABA-responsive downstream genes including *MYB78*, *MYB3*, *PLP3*, *AMY1*, and *RGL2*. These results demonstrate that NAC103 positively regulates ABA response in Arabidopsis.

Keywords NAC103 · ABA response · Seed germination · Seedling growth

Introduction

The NAC (NAM, ATAF1,2, and CUC2) family is one of the largest gene families in plants genome (Olsen et al. 2005). Totally there are 117 NAC transcription factors in Arabidopsis, which are involved in diverse developmental programs and environmental stress responses (Shahnejat-Bushehri et al. 2016). Recently, NAC transcription factors have been found to play important roles in response to endoplasmic reticulum (ER) stress, in which the transcription factor *NAC103*, and the membrane-associated transcription factors *NAC089* and *NAC062* are all up-regulated by ER stress. NAC062 relocates from the plasma membrane to the nucleus and regulates the expression of ER stress-responsive genes (Yang et al. 2014a), and NAC089 relocates from the ER membrane to the nucleus to induce programmed cell death (PCD) (Yang et al. 2014b) following ER stress treatment. NAC103 is a typical NAC family transcription factor with its transcriptional activation domain located in the C-terminus. NAC103 localizes in the nucleus and regulates UPR downstream genes including *CRT1*, *CNX1*, *PDI5* and *UBC32* (Sun et al. 2013). Recently, NAC103 was reported to be involved in the expression of genes related to DNA damage response (Ryu et al. 2019).

Abscisic acid (ABA) is a kind of plant hormone widely existing in plants, which is involved in regulating the growth and development of plants, such as seed dormancy, seed

Communicated by Fuminori Takahashi.

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s00425-020-03502-2) contains supplementary material, which is available to authorized users.

✉ Ling Sun
sunling090909@163.com

✉ Mei-Jing Wang
wangmeijing@zju.edu.cn

[1] School of Food and Biological Engineering, Jiangsu University, Zhenjiang, Jiangsu, China

[2] State Key Laboratory of Plant Physiology and Biochemistry, College of Life Sciences, Zhejiang University, Hangzhou, Zhejiang, China

germination, seedling growth, leaf senescence, and other physiological processes (Seok et al. 2017; Pelagio-Flores et al. 2019). ABA is also a stress hormone that is rapidly accumulated in response to biotic and abiotic stresses. Seed dormancy is very important for the survival of plants. It can ensure that seeds germinate only when the external environment conditions are suitable. At present, many transcription factor families have been reported to play regulatory roles in the ABA pathways, such as B3, AP2/ERF, bZIP (Zhou et al. 2015). ABI3 (Giraudat et al. 1992), ABI4 (Finkelstein 1994; Finkelstein et al. 1998; Finkelstein and Lynch 2000), and ABI5 (Finkelstein 1994; Finkelstein and Lynch 2000) are all positive regulators in the process of ABA signal transduction and their mutants are less sensitive to ABA in the process of seed germination and seedling growth. In addition, some members of other transcription factor families were also found to participate in ABAresponses, such as MYB/MYC (bHLH), NAC, HD-Zip and WRKY families. At least 31 members in the NAC family are strongly up-regulated by ABA or other environmental stresses. Among them, ANAC019 (At1g52890) (Jensen et al. 2010), ANAC055 (At3g15500), ANAC072 (At4g27410) (Tran et al. 2004) and VNI2/ANAC083 (At5g13180) (Yang et al. 2011), have been demonstrated to be positive regulators in the ABA signaling pathway. However, the role of NAC103 in the ABA pathway has not yet been reported.

In the current study, we examined the biological function of NAC103 in ABA responses during seed germination and seedling growth. Genetic experiments demonstrated that NAC103 is a positive regulator in the ABA pathway. RNA-seq and qRT-PCR results showed that NAC103 regulates several ABA-responsive downstream genes. Furthermore, NAC103 is induced by ABA treatment at both transcriptional and post-translational levels. Thus, NAC103 plays an important role in ABA responses, especially during seed germination.

Materials and methods

Plant materials and growth conditions

All Arabidopsis wild-type (WT), *nac103* gene-edited mutants obtained by CRISPR-Cas9 system and NAC103 overexpression plants (Sun et al. 2013) in this study were in the Columbia (Col-0) ecotype background. Seeds were surface-sterilized for 5 s in 50% ethanol and for 10 min in 0.15% NaClO solution and then rinsed three times with sterile water. The seeds were cold-treated for 3 days at 4 °C in the dark and grown on agar plates containing half-strength MS (1/2 MS) medium including multiple vitamins, 0.05% MES buffer and 1.2% sucrose in an illuminated growth chamber with long-day conditions of 16 h light and 8 h dark at 22 °C.

Seed germination and seedling greening ratio analyses

Seeds were surface-sterilized and plated on 1/2 MS medium with different concentrations of ABA (Sigma, America) as shown in the experiments. Emergent radicles were counted at the indicated time points. The averaged seed germination ratio was obtained from three experimental replicates and about 50 seeds per each genotype were observed. Seedling greening ratio was obtained by counting the number of germinated seeds with green seedlings divided by the total number of tested seeds.

Plasmid construction for CRISPR-Cas9 system

According to the online search software (https://crispr.mit.edu/), the mutation targets (target 1:CCATTGCTGAGG TCGACATTTAC; target 2:TGTGGTTACTGGAAGACC ACAGG) with high scores were found in sequences encoding the NAC domain of NAC103. The target 1 or 2 was constructed on AtU6-26-sgRNA-SK plasmid vector using *Bsa*I enzyme, and the fragments recovered by *Nhe*I and *Spe*I were used as sgRNA cassette. At 37 °C, pCAMBIA1300-pYAO:Cas9 plasmids were digested with *Spe*I (Yan et al. 2015), and then dephosphorylated with alkaline phosphatase, connected with sgRNA cassette with T4 ligase, and a binary carrier with a single target was obtained. Binary vector containing target 1 digested with *Spe*I single enzyme was used as the carrier, the fragments from the binary vector containing target 2 digested with *Nhe*I and *Spe*I double enzyme were ligated into the carrier to form the binary vector containing two targets, which were transferred into *Arabidopsis thaliana* with the floral-dip method, as described previously (Clough and Bent 1998). Transgenic Arabidopsis seeds were planted on the 1/2 MS plates containing hygromycin (25 mg/L) to screen transgenic plants. Fragment of *NAC103* gene was amplified by PCR and sequenced in T0–T2 generation transgenic plants. The sequencing results were compared with the information on the website of NCBI (www.ncbi.nlm.nih.gov) to screen for *nac103* mutants.

RNA sequencing and gene expression analyses

About 7-day-old seedlings grown on 1/2 MS plates treated with or without 200 μM ABA were immediately collected for RNA-seq. Three biological replicates for each sample were used in this study. The total messenger RNA (mRNA) of the sample was extracted using mirVana™ miRNA Isolation Kit (Ambion-1561, Invitrogen, America) according to

the manufacturer's instructions. The cDNA libraries were constructed using multiple kits according to the manufacturer's instructions. After the cDNA libraries passed the quality test in a bioanalyzer (Agilent 2100, America), then they were sequenced on the Illumina sequencing platform (HiSeq X Ten, America). Raw RNA-seq data were processed using Trimmomatic (Bolger et al. 2014). The clean reads were obtained by removing the reads containing ploy-N and the low-quality reads, and then mapped to Arabidopsis reference genome TAIR10.31 (ftp://ftp.ensemblgenomes.org/pub/plants/release-31/fasta/arabidopsis_thaliana/dna/Arabidopsis_thaliana.TAIR10.31.dna.genome.fa.gz) using hisat2 software (Kim et al. 2015). The gene expression was calculated by an fpkm method (Adam et al. 2011) (fragments per kb per million reads), which was the number of fragments per thousand base length of a gene per million fragments. The number of counts of each sample gene was standardized by DESeq (Anders et al. 2012) software (base mean value was used to estimate the expression amount), and the analysis of different expression genes (DEGs) was calculated based on fold change and *p* value. All of the raw sequencing data were deposited in the National Center for Biotechnology Information (NCBI). The related raw data were as follows: BioProject ID: PRJNA643876, BioSample accession: SAMN15437556, and SRA accession: SRR12158819-30. To analyze the gene expression, quantitative RT-PCR was performed to validate the accuracy of the RNA-seq data (Sun et al. 2013). 2 µg total RNA was used for the first-stand cDNA synthesis using PrimeScript RT Master mix (RR036A, TaKaRa, Japan) according to the manufacturer's instructions. *Actin* and *UBQ5* were used as internal controls for normalization in quantitative reverse transcription polymerase chain reaction (qRT-PCR) analyses. The primers used in this study were given in Table S1.

Results

NAC103 is induced by ABA at both transcriptional and protein levels

Previous studies showed that many ER stress regulators, such as S2P, are involved in ABA responses (Zhou et al. 2015), we were interested in the possible role of NAC103 in ABA response. The effects of ABA on the transcriptional expression of *NAC103* were analyzed. Results showed that the transcriptional expression of *NAC103* was up-regulated by ABA treatment in wild-type seedlings, compared with the expression of *NAC103* under normal growth conditions (Fig. 1a). Moreover, the protein level of NAC103-YFP in *NAC103* over-expressing seedlings (CaMV35S::NAC103-YFP) was also increased under ABA treatment conditions

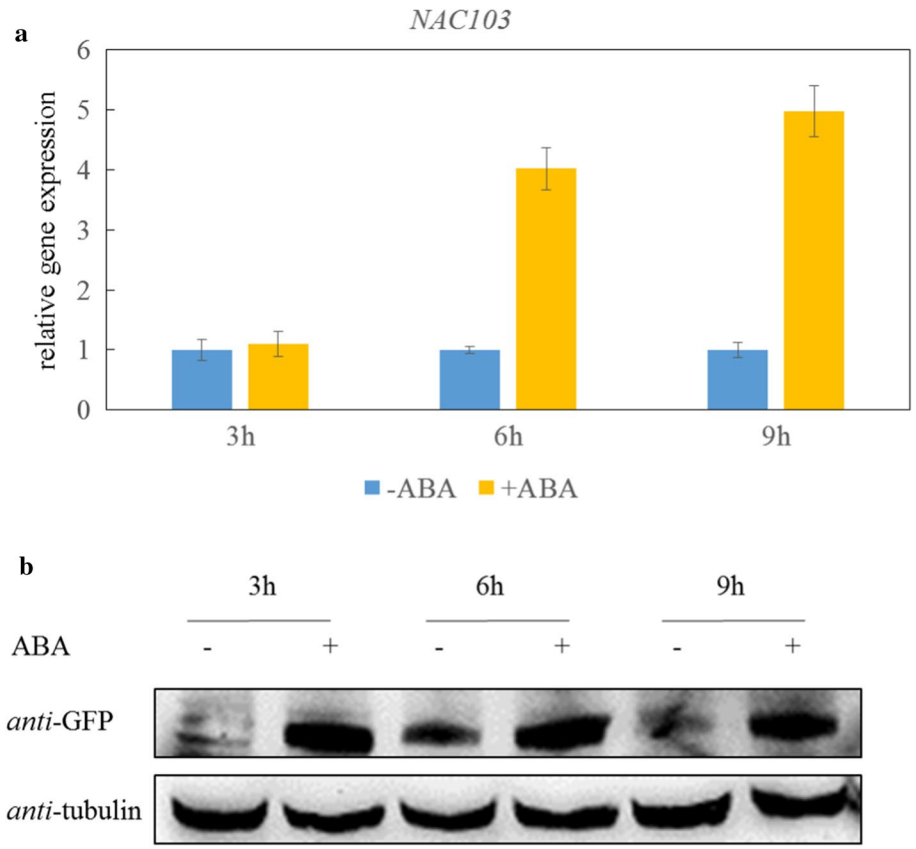

Fig. 1 NAC103 is induced by ABA. **a** Up-regulation of *NAC103* in the wild-type seedlings (8 days after stratification) by ABA treatment. Relative gene expression is the value of wild-type plants treated with ABA (200 µM, 3 h, 6 h or 9 h) divided by that of non-treated wild-type plants, both of which were normalized to the expression of *Actin*. Bars depict SEM ($n=3$). **b** Protein accumulation of NAC103 following ABA treatment. NAC103-YFP was detected by western blot with *anti*-GFP in transgenic plants (CaMV35S::NAC103-YFP) (8 days after stratification) treated or untreated with ABA (200 µM) for the indicated processing time. Anti-tubulin was used as a loading control

(Fig. 1b). Therefore, NAC103 was induced by ABA at both transcriptional and protein levels.

Generation of *nac103* loss-of-function mutants using the CRISPR-Cas9 gene-editing system

To analyze the molecular function of NAC103 in the ABA pathway, we used CRISPR-Cas9 system to generate *NAC103* loss-of-function mutants. After primary screening and secondary screening, we obtained two independent mutant lines, *nac103-1* and *nac103-2*, in which the NAC domains of NAC103 are predicted to be truncated (Fig. S1). In *nac103* mutants, either a nucleotide T or a G was inserted after 115 bases from the transcription initiation site, resulting in an open reading frame-shift and premature termination (Fig. S1B, C). Homozygous mutants were screened and used for subsequent functional analysis.

NAC103 is a positive regulator of ABA response during seed germination

To analyze the biological function of NAC103 under normal soil-grown conditions, phenotypic differences between the *nac103* mutants and wild-type plants were compared (Fig. S2). Compared with the wild-type Arabidopsis, the *nac103* mutants had no significant difference at seedling stage as well as at reproductive stage (Fig. S2). To analyze the biological function of NAC103 in ABA responses, the ABA sensitivity of wild-type, *NAC103* overexpression plants (NAC103-OE1, NAC103-OE3), and *nac103* mutant plants (*nac103-1*, *nac103-2*) were compared. The seed germination rates of these materials were examined on 1/2 MS plates supplemented without or with 0.5 μM, 1.0 μM, 1.5 μM, 3.0 μM ABA. On the 1/2 MS plates without additional ABA, the wild-type, NAC103-OE1 and NAC103-OE3 showed similar germination rates (85–90%) after 48 h of germination (Fig. 2). When 0.5 μM, 1.0 μM, 1.5 μM or 3.0 μM ABA was added, the seed germination rates of NAC103-OE1 and NAC103-OE3 were lower than that of the wild-type plants within 72 h, especially when 1.5 μM or 3.0 μM ABA was added (Fig. 2). Without adding ABA, *nac103-1* and *nac103-2* showed almost the same germination rates as WT during germination (Fig. 3). When 0.5 μM, 1.0 μM, 1.5 μM or 3.0 μM ABA was added, the germination rates of *nac103-1* and *nac103-2* were higher than that of the wild-type Arabidopsis plants at different time points, especially when the concentration of ABA was 3.0 μM, the germination rates of *nac103-1* and *nac103-2* were 38.9% and 29.6%, respectively, which were significantly higher than that of wild-type Arabidopsis (only 4.4%) (Fig. 3). These results demonstrated that NAC103 is a positive regulator of ABA response during seed germination.

NAC103 regulates ABA response during seedling growth

To study the function of NAC103 in regulating seedling growth under high ABA conditions, seedling greening ratio of the wild-type, *NAC103* overexpression plants (NAC103-OE1, NAC103-OE3), and *nac103* mutants (*nac103-1*, *nac103-2*) were examined. As shown in the Fig. 4, on the 1/2 MS plates without ABA, the tested plants had similar growth rates and the seedling greening ratios were more than 97%; when 0.3 μM, 0.5 μM, or 0.7 μM ABA was added to the growth medium, the *nac103-1* and *nac103-2* mutants had similar seedling greening ratios to that of the wild-type plants, however, seedling greening ratios of NAC103-OE1 and NAC103-OE2 plants were significantly lower than that of the wild-type plants. These results suggested that overexpression of *NAC103* inhibited seedling growth when plants were treated with ABA. The *nac103* mutants had no obvious seedling growth phenotype under ABA treatment, most possibly due to the potential functional redundancy of other ABA pathways in Arabidopsis.

NAC103 regulates ABA downstream gene expression

To further analyze the biological mechanism of NAC103 in ABA response, seedlings of the wild-type and *nac103-1* mutants were treated without or with ABA (200 μM) for 5 h and sampled for RNA-seq analyses. The sequencing depth, the gene annotations and counts of genes mapped on the Arabidopsis genome were analyzed for each biological sample (Table S2–3), and there was a significant correlation among the three replicates of each biological sample. The differential expression profiles of different samples were obtained (Fig. 5). Among the differential expression genes (DEGs), 2395 genes were up-regulated by ABA treatment both in the wild-type and *nac103-1* mutants, 1016 genes were specifically up-regulated in *nac103-1* mutants while 289 genes were specifically up-regulated in wild-type plants. In contrast, there were 1932 down-regulated DEGs both in the wild-type and *nac103-1* mutants, 1012 down-regulated DEGs only in *nac103-1* mutants, and 699 down-regulated DEGs only in the wild-type plants.

Based on the published results (Sun et al. 2013), *NAC103* is a transcription factor with transcriptional activation activity. To explore downstream genes regulated by NAC103 in the ABA pathway, we focused on the specific up-regulated DEGs in wild-type Arabidopsis, and the common up-regulated DEGs with 1.5-fold higher ratio in the WT than that in the *nac103-1* plants, and GO and KEGG function enrichment was analyzed. The results showed that many of the DEGs were concentrated in terpenoid anabolism, ABA synthesis and metabolism, and

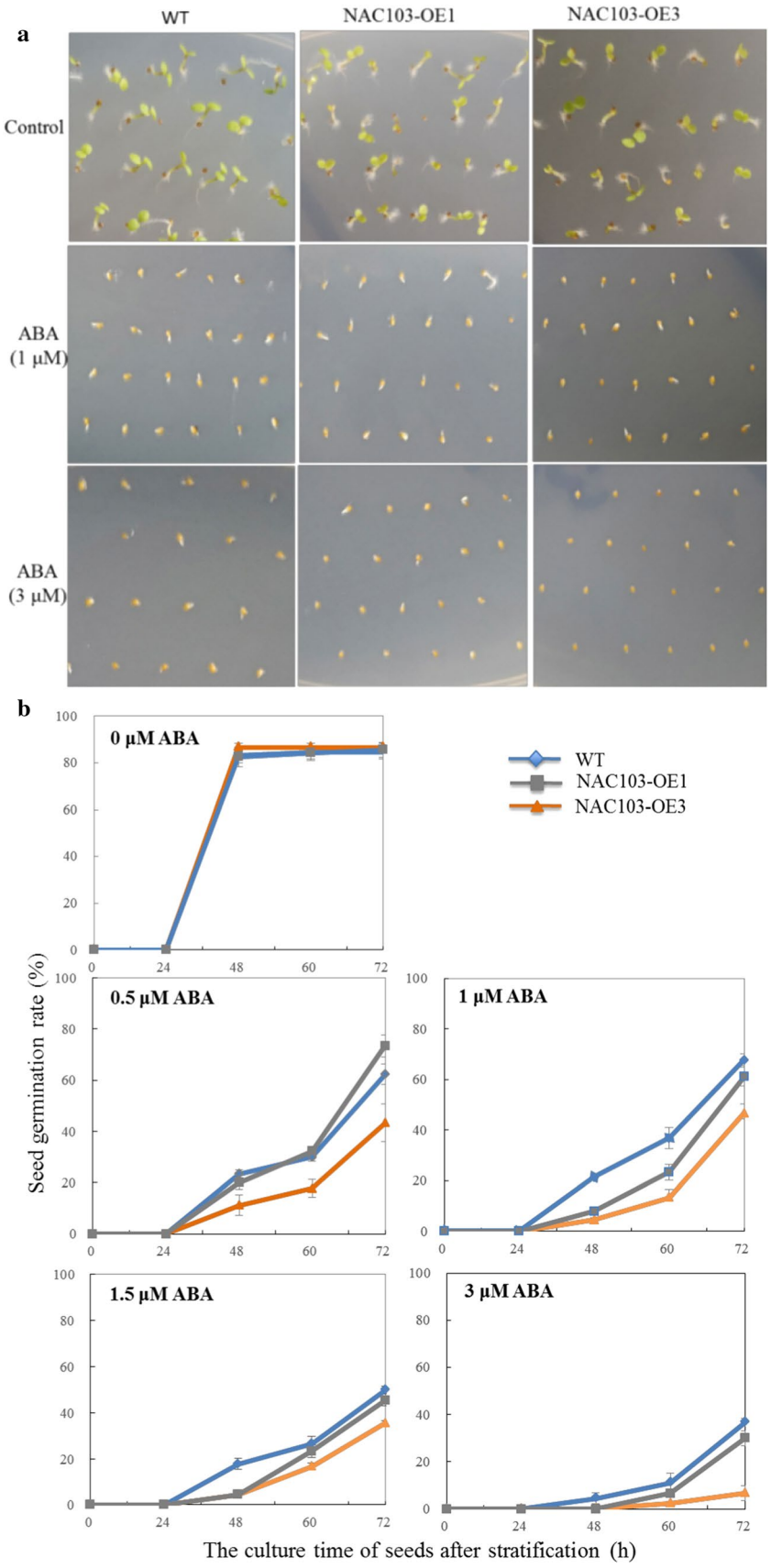

Fig. 2 The *NAC103* overexpression plants are more sensitive to ABA during seed germination. **a**, **b** Seed germination image (**a**) and statistical seed germination ratio analysis (**b**) of wild-type (WT) and *NAC103* overexpression plants on the 1/2 MS plates with or without ABA at indicated time. Representative photographs (**a**) were taken at 3 day post-imbibition (dpi). Radicle emergence of seeds is defined as germination. Emergent radicles were counted at the indicated time points post-imbibition. The averaged seed germination ratio was obtained from three experimental replicates and about 50 seeds per each genotype were observed. Bars depict SEM ($n = 3$)

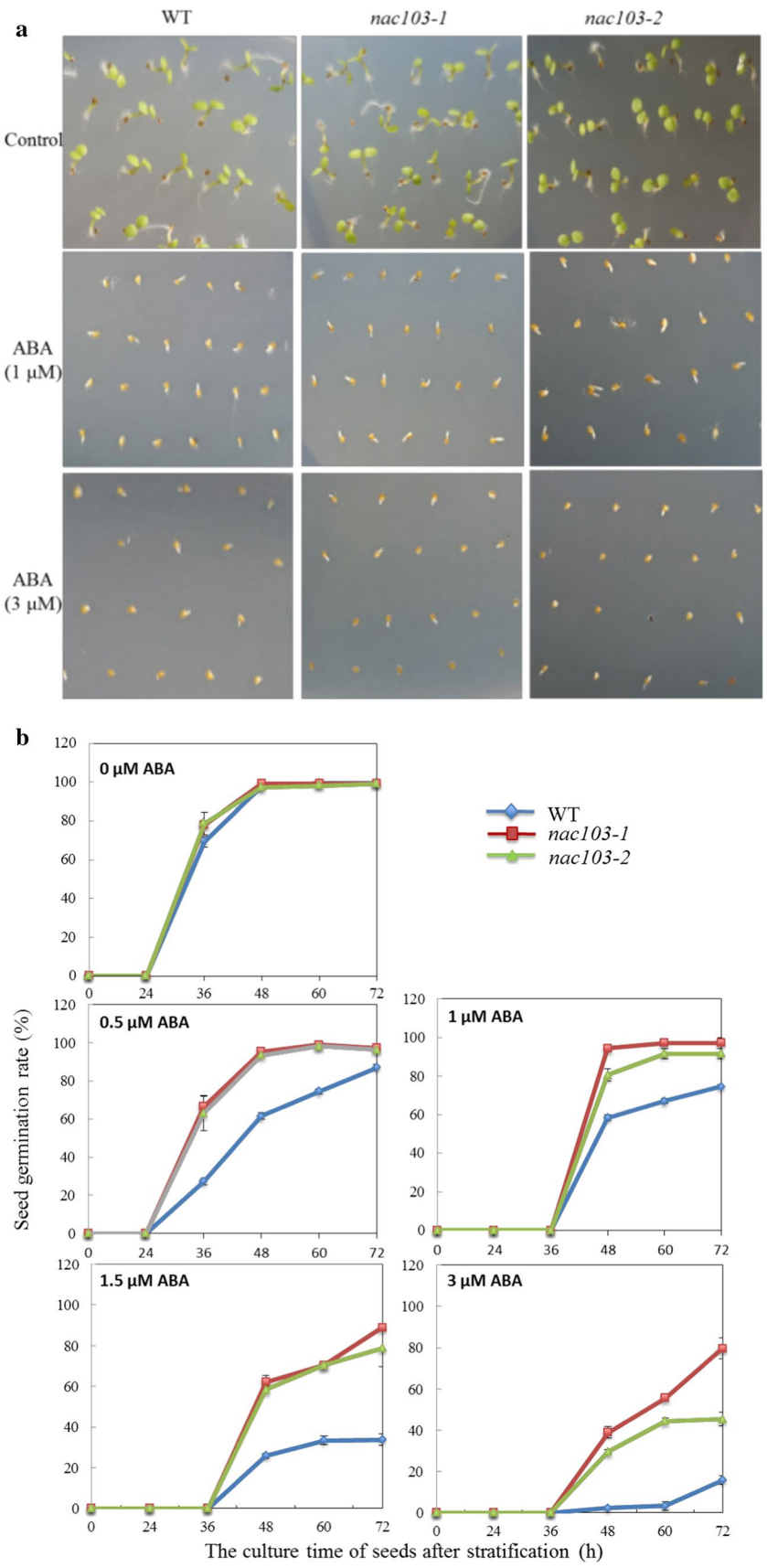

Fig. 3 The *nac103* mutant plants are less sensitive to ABA during seed germination. **a**, **b** Seed germination image (**a**) and statistical seed germination ratio analysis (**b**) of wild-type (WT) and *nac103* gene-edited mutants on the 1/2 MS plates with or without ABA at indicated time. Representative photographs (**a**) were taken at 3 day post-imbibition (dpi). Radicle emergence of seeds is defined as germination. Emergent radicles were counted at the indicated time points post-imbibition. The averaged seed germination ratio was obtained from three experimental replicates and about 50 seeds per each genotype were observed. Bars depict SEM ($n = 3$)

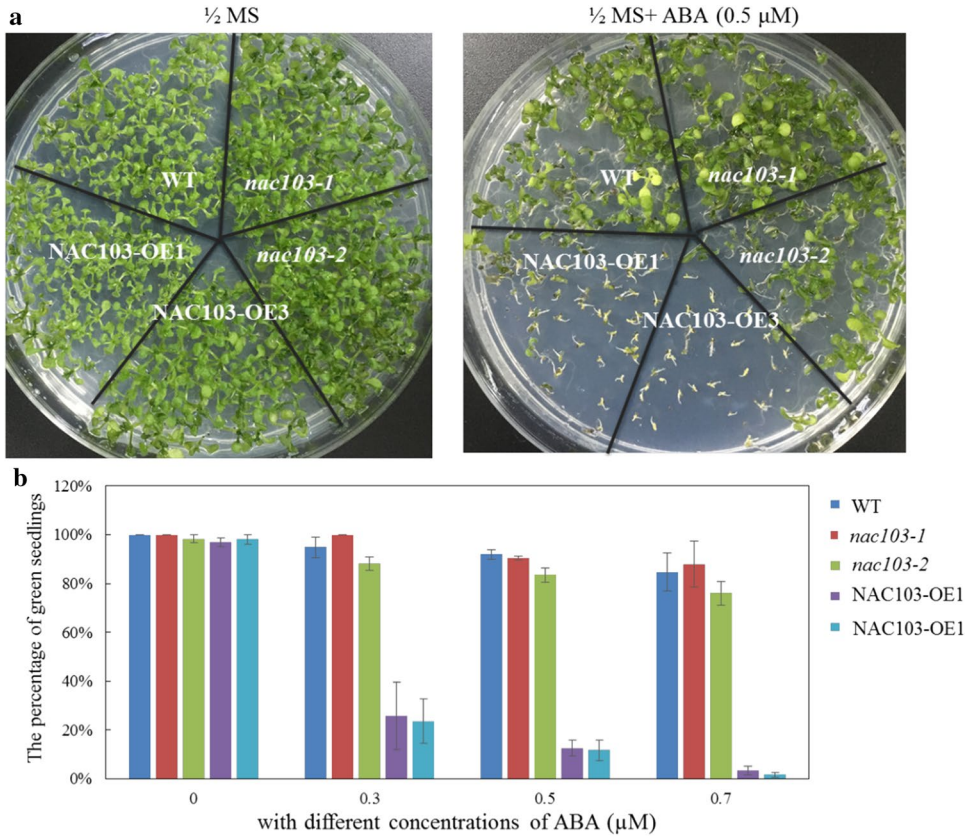

Fig. 4 The *NAC103* overexpression plants are more sensitive to ABA during seedling growth. **a**, **b** The phenotype of the wild-type (WT), *nac103 mutants* and *NAC103* overexpression plants (25 days after stratification) on the 1/2 MS with or without ABA (**a**). The percentage of green seedlings were calculated on the plates supplemented with different concentrations of ABA (**b**). Bars depict SEM ($n = 3$)

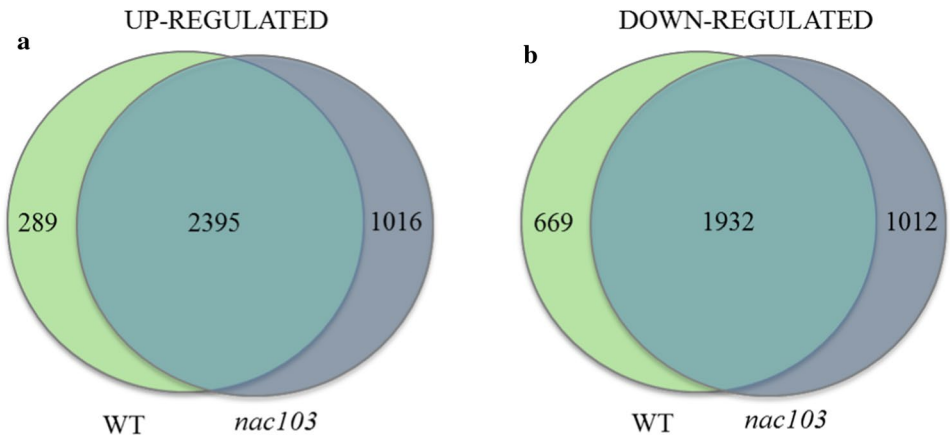

Fig. 5 NAC103 regulates ABA-responsive genes. **a**, **b** The differentially up-regulated (**a**) and down-regulated (**b**) genes by ABA treatment in the wild-type (WT) and *nac103-1* mutant plants (8 days after stratification)

gibberellin metabolism. Most importantly, DEGs related to ABA responses and ABA metabolism were enriched in the NAC103-regulated downstream genes (Table 1). The relative expression of *RGL2*, *ICMEL2* and *NCED9* in the wild-type and *nac103-1* mutant treated with or without ABA were validated by qRT-PCR method (Fig. 6). The results showed that the gene expression of *RGL2*, *ICMEL2* and *NCED9* increased after ABA treatment in the wild-type Arabidopsis, in contrast, the gene expression of *RGL2*, *ICMEL2* and *NCED9* did not increase after ABA treatment in the *nac103-1* mutant, indicating that these genes might be regulated by NAC103 in response to ABA treatment.

Discussion

Recently, several studies showed that plant-specific NAC103 has a variety of biological functions in various stress responses. In 2013, Arabidopsis NAC103 was first proved to be a transcription factor with transcriptional activation

Table 1 NAC103 regulates ABA-responsive genes

Name	Gene	FC in WT	*p* value	FC in *nac103-1*	*p* value	Description
PLP3	AT4G37050	226.5	0.000	34.7	0.000	Patatin-like protein 3
AMY1	AT4G25000	104.4	0.000	54.7	0.000	Alpha-amylase 1
HVA22B	AT5G62490	28.6	0.000	7.7	0.023	HVA22-like protein b
DUF1264	AT1G05510	23.7	0.001	10.5	0.008	Protein of unknown function
ICMEL2	AT3G02410	8.4	0.000	4.2	0.000	Probable isoprenylcysteine alpha-carbonyl methylesterase
MYB78	AT5G49620	5.5	0.024	2.6	0.140	myb domain protein 78
MYB3	AT1G22640	4.6	0.000	3.2	0.000	Transcription factor MYB3
ATDI21	AT4G15910	4.5	0.000	3.1	0.000	Drought-induced 21
DOG1	AT5G45830	3.5	0.000	2.3	0.000	Delay of germination 1
PCMP-E32	AT5G08490	2.6	0.004	1.7	0.146	Putative pentatricopeptide repeat-containing protein At5g08490
ETC3	AT4G01060	2.5	0.003	1.7	0.076	MYB-like transcription factor ETC3
RGL2	AT3G03450	2.0	0.001	1.1	0.557	DELLA protein RGL2
NCED9	AT1G78390	36.7	0.000	8.2	0.001	9-*cis*-epoxycarotenoid dioxygenase NCED9
AAO4	AT1G04580	8.1	0.014	3.9	0.033	Benzaldehyde dehydrogenase (NAD(+))

FC fold change

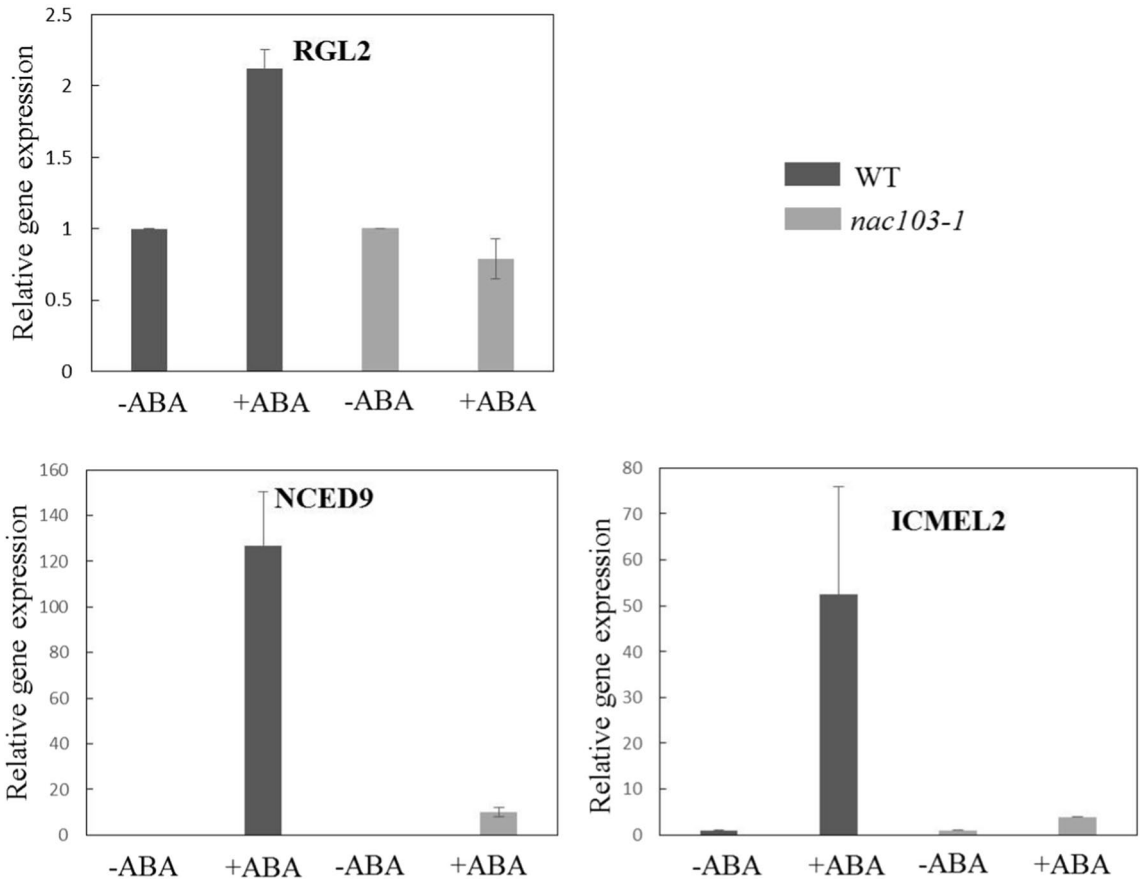

Fig. 6 Validation of RNA-seq by qRT-PCR analysis of the NAC103-regulated ABA-responsive downstream genes. Relative gene expression is the value of wild-type or *nac103-1* mutant plants treated with ABA (200 μM, 5 h) divided by that of non-treated wild-type or *nac103-1* mutant plants (8 days after stratification), which were normalized to the expression of actin. Bars depict means SEM ($n = 3$)

activity (Sun et al. 2013). The expression of *NAC103* is induced by ER stress, which is directly regulated by bZIP60 through recognizing the UPRE-III *cis*-acting elements. NAC103 protein enters nucleus to regulate UPR downstream genes, such as *BiP3*, *CRT1*, *CNX1*, *PDI5* and *UBC32*. Overexpression of NAC103 significantly increased the transcription of ER stress-responsive (ERSR) genes, such as *CRT1*, *CNX1*, *PDI5* and *UBC32* as well as DDR genes. Interestingly, *NAC103* was also induced by genotoxic reagent during DNA replication stress. In 2019, Tae Ho Ryu et al. (2019) reported that NAC103 was involved in DNA damage response (DDR) downstream of SOG1, a master regulator in genotoxic stress response. The *nac103* mutants displayed substantial inhibition of DDR gene expression after gamma radiation or radiomimetic zeocin treatment, however, overexpression of *NAC103* increased DDR gene expression without genotoxic stress and substantially rescued the phenotypic changes in the *sog1-1* mutant after zeocin treatment. It is known that unmitigated ER stress also induces programed cell death (PCD) (Yang et al. 2014b). Since genotoxic reagents also trigger PCD. Therefore, during ER stress response and DDR, NAC103 may mediate or inhibit PCD in Arabidopsis. In this study, the Arabidopsis NAC103 was shown to be induced by ABA treatment at the transcriptional and protein level. Under the exogenous ABA, overexpression of *NAC103* inhibited seed germination rates and seedling growth, in contrast, *nac103* mutants displayed increased seed germination rates. Moreover, NAC103 regulated several ABA downstream gene expressions as revealed by RNA-seq and qRT-PCR. In Canola (*Brassica Napus* L.), BnaNAC103 (Niu et al. 2014) was reported to respond to multiple stress signals, including cold, salicylic acid (SA) and fungal pathogen *Sclerotinia sclerotiorum*, and reactive oxygen species (ROS). In conclusion, the plant-specific NAC103 plays important roles in response to multi-stresses including ABA, ER stress and DNA damage. ABA, ER stress and DNA damage are all closely related to various environmental stresses. ABA, as a hormone widely found in plants, is involved in the regulation of plant development and plant responses to adversity stress, such as drought, high salt, low temperature. Seki et al. (2002) identified 245 ABA-induced genes in *Arabidopsis thaliana*, among which 155 genes (63%) were induced by drought, 133 genes (52%) by high salinity, and only 25 genes (10%) by low temperature. Moreover, 114 genes (46%) were found to be induced by both drought and high salt, while only 20 (8%) were induced by both drought and low temperature. ER stress is induced in plants during certain developmental stages or under adverse environmental conditions, due to the accumulation of unfolded or misfolded proteins in the ER (Liu et al. 2016). Among the ER stress-induced genes, bZIP17 was also induced by salt stress (Liu et al. 2008), bZIP28 (Deng et al. 2011) and bZIP60 (Gao et al. 2008) were involved in high-temperature stress, and IRE1A and IRE1B were both induced by pathogen infection and salicylic acid (Humbert et al. 2012). bZIP60, bZIP17, S2P and IRE1, the important regulators in ER stress response, are all involved in ABA responses (Liu et al. 2008; Zhou et al. 2015; Xian et al. 2016). Although it is not clear how ABA responses links to ER stress responses, it is possible that ABA treatment induces a subset of proteins that has some protein folding defects in the ER, or ABA treatment affects the stability of ER membranes. DNA damage is caused by abiotic and biotic environmental stresses, including UV-B, ozone, pathogens and endogenous metabolic processes, by generating reactive oxygen species (ROS) in plant cells (Rold and Ariza 2009). Because ABA, ER stress and DNA damage are closely related to environmental stress, many genes, such as NAC103, bZIP17, bZIP28, bZIP60, have various functions in response to multi-stresses.

NAC family, as one of the largest plant-specific families, is involved in diverse developmental and signaling events. NAC transcription factors in ABA signaling have been gradually revealed. The Arabidopsis NAC Transcription Factor ANAC096 (Xu et al. 2013) is involved in a synergistic relationship with a subset of bZIP-type transcription factors, ABRE-binding factor and ABRE-binding protein (ABF/AREB) for the transcriptional activation of ABA-inducible genes in response to dehydration and osmotic stresses. The transcript of NAC072 (Li et al. 2016) is upregulated by ABF3 in ABA response, and NAC072 protein interacts with ABF3. NAC072 and ABF3 cooperate to regulate *RD29A* expression, but are antagonistic when regulating RD29B expression. Therefore, NAC072 displays a dual function in ABF3-mediated ABA-responsive gene regulation. VND-INTERACTING2 (VNI2) (Yang et al. 2011), an ABA-responsive NAC transcription factor, integrates ABA-mediated abiotic stress signals into leaf aging by regulating a subset of COLD-REGULATED (COR) and RESPONSIVE TO DEHYDRATION (RD) genes. In this study, we demonstrate that NAC103 was induced by ABA at transcriptional and protein levels (Fig. 1). Overexpression of NAC103 inhibited seed germination and seedlings growth under exogenous ABA treatment (Fig. 2), but mutation of *nac103* increased seed germination (Fig. 3), which suggests that NAC103 is a positive regulator in ABA response.

Through RNA-seq analysis, we found that NAC103 was involved in the regulation of some ABA-related gene expression, including *PLP3* (Patatin-like protein 3), *AMY1* (Alpha-amylase 1) (Wang et al. 2009), *HVA22B* (HVA22-like protein b), *RGL2* (DELLA protein RGL2) (Liu et al. 2016), MYB family transcription factor *MYB78* and *MYB3* (Monika et al. 2018), and *ETC3* (MYB-like transcription factor ETC3). The expression of these genes was significantly induced by ABA in the wild-type, however, the fold change was decreased in the *nac103* mutants, which suggests

that NAC103 partially regulates the expression of these genes (Table1; Fig. 6). Although the gene expression analysis was done with seedlings rather than germinating seeds due to technical difficulties, the NAC103-dependent genes identified in this study may also be important for seed germination in response to ABA treatment, since some of these downstream genes have already been reported to be involved in ABA responses during seed germination. It was reported that there was interaction between ANAC (abscisic acid-responsive NAC) proteins and RING-H2 (Greve et al. 2003). Both RHA2a (the small RING-H2 protein) and ANAC have nuclear localization signal (NLS) to enter the nucleus, which suggests that the interaction between these two proteins regulates downstream genes expression. Moreover, the conserved N-terminal NAC domains of NAC proteins consist of a twisted β-sheet surrounded by a few helical elements, but not possess a classical helix–turn–helix motif, which can bind both DNA and other proteins (Ernst et al. 2004). Therefore, the structure of NAC103 in the N-terminus may contribute to the regulation of the ABA downstream genes.

Author contribution statement LS, M-JW and J-XL conceived and designed research. LS, L-PL, M-JW, Y-ZW and LY conducted experiments. LS, L-PL, and M-JW analyzed data. LS wrote the manuscript. J-XL and M-JW revised the manuscript. All authors read and approved the manuscript.

Acknowledgements This study was financially supported by grants from the National Natural Science Foundation of China (31600197 and 32000374) and the Startup Scientific Research Fund from Jiangsu University for Advanced Professionals (14JDG158). The authors also thank Dr. Yu Wang, Jia-Jia Han and Sun-Jie Lu for their help in gene-editing.

Compliance with ethical standards

Conflict of interest We declare that we have no financial and personal relationships with other people or organizations that can inappropriately influence our work, there is no professional or other personal interest of any nature or kind in any product, service and/or company that could be construed as influencing the position presented in, or the review of, the manuscript entitled, "NAC103, a NAC family transcription factor, regulates ABA response during seed germination and seedling growth in Arabidopsis".

References

Adam R, Cole T, Julie D, John LR, Lior P (2011) Improving RNA-Seq expression estimates by correcting for fragment bias. Genome Biol 12:R22

Bolger AM, Lohse M, Usadel B (2014) Trimmomatic: a flexible trimmer for illumina sequence data. Bioinformatics 30:2114

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

Deng Y, Humbert S, Liu JX, Srivastava R, Rothstein SJ, Howell SH (2011) Heat induces the splicing by IRE1 of a mRNA encoding a transcription factor involved in the unfolded protein response in Arabidopsis. Proc Natl Acad Sci 108:7247–7252

Ernst HA, Olsen AN, Larsen S, Lo Leggio L (2004) Structure of the conserved domain of ANAC, a member of the NAC family of transcription factors. EMBO Rep 5:297–303

Finkelstein RR (1994) Mutations at two new Arabidopsis ABA response loci are similar to the abi3 mutations. Plant J 5:765–771

Finkelstein RR, Lynch TJ (2000) The Arabidopsis abscisic acid response gene ABI5 encodes a basic leucine zipper transcription factor. Plant Cell 12:599–609

Finkelstein RR, Ming LW, Lynch TJ, Goodman RHM (1998) The Arabidopsis abscisic acid response locus *ABI4* encodes an APETALA 2 domain protein. Plant Cell 10:1043–1054

Gao HB, Brandizzi F, Benning C, Larkin RM (2008) A membrane-tethered transcription factor defines a branch of the heat stress response in *Arabidopsis thaliana*. Proc Natl Acad Sci 105:16398–16403

Giraudat J, Hauge BM, Valon C, Smalle J, Parcy F, Goodman HM (1992) Isolation of the Arabidopsis *ABI3* gene by positional cloning. Plant Cell 4:1251–1261

Greve K, La Cour T, Jensen MK, Poulsen FM, Skriver K (2003) Interactions between plant RING-H2 and plant-specific NAC (NAM/ATAF1/2/CUC2) proteins: RING-H2 molecular specificity and cellular localization. Biochemical J 371:97–108

Humbert S, Zhong SH, Deng Y, Howell SH, Rothstein SJ (2012) Alteration of the bZIP60/IRE1 pathway affects plant response to ER stress in *Arabidopsis thaliana*. PLoS ONE 7:e39023

Jensen MK, Kjaersgaard T, Nielsen MM, Galberg P, Petersen K, O'Shea C, Skriver K (2010) The *Arabidopsis thaliana* NAC transcription factor family: structure–function relationships and determinants of ANAC019 stress signalling. Biochem J 426:183–196

Kim D, Langmead B, Salzberg SL (2015) HISAT: a fast spliced aligner with low memory requirements. Nat Methods 12:357–360

Li X, Li X, Li M, Yan Y, Liu X, Li L (2016) Dual function of NAC072 in ABF3-mediated ABA-responsive gene regulation in Arabidopsis. Front Plant Sci 7:1075–1084

Liu JX, Howell SH (2016) Managing the protein folding demands in the endoplasmic reticulum of plants. New Phytol 211:418–428

Liu JX, Srivastava R, Howell SH (2008) Stress-induced expression of an activated form of AtbZIP17 provides protection from salt stress in Arabidopsis. Plant Cell Environ 31:1735–1743

Liu X, Hu P, Huang M, Tang Y, Li Y, Li L, Hou X (2016) The NF-YC–RGL2 module integrates GA and ABA signalling to regulate seed germination in Arabidopsis. Nat Commun 7:12768–12782

Monika D, Sarika S, Sneha T, Rao R, Kishor G (2018) Transcriptome analysis reveals interplay between hormones, ROS metabolism and cell wall biosynthesis for drought-induced root growth in wheat. Plant Physiol Biochem 130:482–492

Niu F, Wang B, Wu F, Yan J, Li L, Wang C, Wang Y, Yang B, Jiang YQ (2014) Canola (*Brassica napus* L.) NAC103 transcription factor gene is a novel player inducing reactive oxygen species accumulation and cell death in plants. Biochem Biophys Res Commun 454:30–35

Olsen AN, Ernst HA, Leggio LL, Skriver K (2005) NAC transcription factors: structurally distinct, functionally diverse. Trends Plant Sci 10:79–87

Pelagio-Flores R, Munoz-Parra E, Barrera-Ortiz S, Ortiz-Castro R, Saenz-Mata J, Ortega-Amaro MA, Jimenez-Bremont JF, Lopez-Bucio J (2019) The cysteine-rich receptor-like protein kinase CRK28 modulates Arabidopsis growth and development and influences abscisic acid responses. Planta 251:2

Roldán-Arjona T, Ariza RR (2009) Repair and tolerance of oxidative DNA damage in plants. Mutat Res 681:169–179

Ryu TH, Go YS, Choi SH, Kim JI, Chung BY, Kim JH (2019) SOG1-dependent NAC103 modulates the DNA damage response as a transcriptional regulator in Arabidopsis. Plant J 98:83–96

Seki M, Ishida J, Narusaka M, Fujita M, Nanjo T, Umezawa T, Kamiya A, Nakajima M, Enju A, Sakurai T, Satou M, Akiyama K, Kazuko YS, Carninci P, Kawai J, Hayashizaki Y, Shinozaki K (2002) Monitoring the expression pattern of around 7,000 Arabidopsis genes under ABA treatments using a full-length cDNA microarray. Funct Integr Genomics 2:282–291

Seok HY, Woo DH, Nguyen LV, Tran HT, Tarte VN, Mehdi SM, Lee SY, Moon YH (2017) Arabidopsis *AtNAP* functions as a negative regulator via repression of *AREB1* in salt stress response. Planta 245:329–341

Shahnejat-Bushehri S, Tarkowska D, Sakuraba Y, Balazadeh S (2016) Arabidopsis NAC transcription factor JUB1 regulates GA/BR metabolism and signalling. Nat Plants 2:16013

Sun L, Yang ZT, Song ZT, Wang MJ, Liu JX (2013) The plant-specific transcription factor *NAC103* is induced by bZIP60 through a new *cis*-regulatory element to modulate the unfolded protein response in Arabidopsis. Plant J 76:274–286

Tran L-SP, Nakashima K, Sakuma Y, Simpson SD, Fujita Y, Maruyam K, Fujita M, Seki M, Shinozaki K, Yamaguchi-Shinozaki K (2004) Isolation and functional analysis of Arabidopsis stress-inducible NAC transcription factors that bind to a drought-responsive *cis*-element in the early responsive to dehydration stress 1 promoter. Plant Cell 16:2481–2498

Wang J, Yu D, Guo XH, Liu X (2009) Arabidopsis AMY1 expressions and early flowering mutant phenotype. BMB Rep 42:101–105

Xian MJ, Zhang SS, Liu JX, Lu SJ (2016) Membrane-associated transcription factor, bZIP60, is activated by ABA and interacts with MYB7 to regulate seed germination in Arabidopsis. J Fudan Univ (Nat Sci) 55:632–641

Xu ZY, Kim SY, Hyeon DY, Kim DH, Dong T, Park Y, Jin JB, Joo SH, Kim SK, Hong JC (2013) The Arabidopsis NAC transcription factor ANAC096 cooperates with bZIP-type transcription factors in dehydration and osmotic stress responses. Plant Cell 25:4708–4724

Yan L, Wei S, Wu Y, Hu R, Li H, Yang W, Xie Q (2015) High-efficiency genome editing in Arabidopsis using YAO Promoter-Driven CRISPR/Cas9 system. Mol Plant 8:1820–1823

Yang SD, Seo PJ, Yoon HK, Park CM (2011) The Arabidopsis NAC transcription factor VNI2 integrates abscisic acid signals into leaf senescence via the COR/RD genes. Plant Cell 23:2155–2168

Yang ZT, Lu SJ, Wang MJ, Bi DL, Sun L, Zhou SF, Song ZT, Liu JX (2014) A plasma membrane-tethered transcription factor, NAC062/ANAC062/NTL6, mediates the unfolded protein response in Arabidopsis. Plant J 79:1033–1043

Yang ZT, Wang MJ, Sun L, Lu SJ, Bi DL, Sun L, Song ZT, Zhang SS, Zhou SF, Liu JX (2014) The membrane-associated transcription factor NAC089 controls ER-stress-induced programmed cell death in plants. PLoS Genet 10:e1004243

Zhou SF, Sun L, Valdes AE, Engstrom P, Song ZT, Lu SJ, Liu JX (2015) Membrane-associated transcription factor peptidase, site-2 protease, antagonizes ABA signaling in Arabidopsis. New Phytol 208:188–197