

RESEARCH PAPER

# **TaABC1, a member of the activity of *bc*<sub>1</sub> complex protein kinase family from common wheat, confers enhanced tolerance to abiotic stresses in *Arabidopsis***

Caixiang Wang, Ruilian Jing\*, Xinguo Mao, Xiaoping Chang and Ang Li

The National Key Facility for Crop Gene Resources and Genetic Improvement, Institute of Crop Science, Chinese Academy of Agricultural Sciences, Beijing 100081, China

\* To whom correspondence should be addressed. E-mail: [jingrl@caas.net.cn](mailto:jingrl@caas.net.cn)

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## Abstract

Abiotic stresses such as drought, salinity, and low temperature have drastic effects on plant growth and development. However, the molecular mechanisms regulating biochemical and physiological changes in response to stresses are not well understood. Protein kinases are major signal transduction factors among the reported molecular mechanisms mediating acclimation to environmental changes. Protein kinase ABC1 (activity of *bc*<sub>1</sub> complex) is involved in regulating coenzyme Q biosynthesis in mitochondria in yeast (*Saccharomyces cerevisiae*), and in balancing oxidative stress in chloroplasts in *Arabidopsis thaliana*. In the current study, TaABC1 (*Triticum aestivum* L. activity of *bc*<sub>1</sub> complex) protein kinase was localized to the cell membrane, cytoplasm, and nucleus. The effects of overexpressing TaABC1 in transgenic *Arabidopsis* plants on responses to drought, salt, and cold stress were further investigated. Transgenic *Arabidopsis* overexpressing the TaABC1 protein showed lower water loss and higher osmotic potential, photochemistry efficiency, and chlorophyll content, while cell membrane stability and controlled reactive oxygen species homeostasis were maintained. In addition, overexpression of TaABC1 increased the expression of stress-responsive genes, such as *DREB1A*, *DREB2A*, *RD29A*, *ABF3*, *KIN1*, *CBF1*, *LEA*, and *P5CS*, detected by real-time PCR analysis. The results suggest that TaABC1 overexpression enhances drought, salt, and cold stress tolerance in *Arabidopsis*, and imply that TaABC1 may act as a regulatory factor involved in a multiple stress response pathways.

**Key words:** Abiotic stress response, biochemical character, gene expression, physiological character, protein kinases, TaABC1.

## Introduction

Plants, as sessile organisms, have evolved appropriate mechanisms to adapt to abiotic stresses, such as drought, high salt, and cold (Fujita *et al.*, 2006). Plants trigger an orchestrated complex network of signal events to protect cellular activities and regulate whole plant physiological and biochemical responses when environmental stresses occur. Numerous stress-induced genes have been identified, including genes that function in initial perception and transmission of stress signals and those subsequently activated to enable the stress response (Ingram and Bartels, 1996; Shinozaki *et al.*, 2003; Bartels and Sunkar, 2005; Yamaguchi-

Shinozaki and Shinozaki, 2006). In diverse signalling pathways, protein phosphorylation is an important mechanism by which plants respond to external stimuli. Although genetic evidence shows that mitogen-activated protein kinase (MAPK) (Jonak *et al.*, 1996; Mizoguchi *et al.*, 1996; Munnik *et al.*, 1999; Ichimura *et al.*, 2000; Knight and Knight, 2001; Singh *et al.*, 2002), calcium-dependent protein kinase (CDPK) (Ludwig *et al.*, 2004), and SNF-1-related protein kinases (SnRKs) (Li *et al.*, 2000; Mustilli *et al.*, 2002; Kobayashi *et al.*, 2004) are activated by abscisic acid (ABA) and osmotic stress, knowledge concerning components

of these pathways, how the different molecules interact with each other, and where they are positioned in the complex signalling network is incomplete.

*ABC1* (activity of *bc*<sub>1</sub> complex) was originally identified as a multicopy suppressor of a cytochrome *b* mRNA translation defect in *Saccharomyces cerevisiae* (Bousquet *et al.*, 1991). Mitochondrial *ABC1* is essential for the proper conformation and activity of the cytochrome *bc*<sub>1</sub> complex III in yeast (Brasseur *et al.*, 1997). *ABC1* was subsequently isolated as *COQ8* and found to be necessary for yeast coenzyme Q (COQ) synthesis (Do *et al.*, 2001). Several studies indicate that yeast *abc1coq8* mutants might be responsible for the respiratory deficiency caused by disruption of COQ biosynthesis (Saiki *et al.*, 2003; Hsieh *et al.*, 2004). Recent evidence showed that a yeast *ABC1* homologue, *p74*, is required for COQ biosynthesis in *Trypanosoma congolense* (Baticados *et al.*, 2005). Although the exact function of the *ABC1/COQ8* protein is unknown, it is classified as a putative protein kinase based on the presence of kinase motifs (Leonard *et al.*, 1998; Poon *et al.*, 2000). *CABC1* (chaperone activity of *bc*<sub>1</sub> complex), a homologue of yeast *ABC1* protein in humans, is involved in apoptosis through a mitochondrial pathway (Iizumi *et al.*, 2002). Mollet *et al.* (2008) demonstrated that *CABC1* mutations form a homogeneous group of ubiquinone deficiencies (Mollet *et al.*, 2008). Plant *ABC1* genes are poorly characterized. In *Arabidopsis*, 17 genes contain typical *ABC1* motifs, but only two genes are described (Cardazzo *et al.*, 1998; Jasinski *et al.*, 2008). The first cloned *ABC1*-like protein partially restored the activity of complex III in an *abc1* mutant (Cardazzo *et al.*, 1998). Another *ABC1* gene, *AtOSAI* (oxidative stress-related *Abc1*-like), involved in balancing oxidative stress, does not complement the phenotype of *abc1* mutation in yeast (Jasinski *et al.*, 2008).

In this study, *TaABC1*, a member of the *ABC1* protein kinase family from wheat, was overexpressed in *Arabidopsis*. Subsequent evidence showed that overexpression of *TaABC1* produces an enhanced tolerance to drought, salt, and cold stress in *Arabidopsis*, and indicated the functional diversity of the *ABC1* protein kinase family.

## Materials and methods

### Plant materials and growth conditions

Seeds of wild-type *Arabidopsis thaliana* (Columbia 0 type) and transgenic *Arabidopsis* were surface-sterilized with 10% bleach and 0.01% Triton X-100, and washed six times with sterile water. Sterile seeds were plated on Murashige and Skoog (MS) medium plus 0.8% (w/v) agar and 3.0% (w/v) sucrose. Plates were placed in darkness for 2 d at 4 °C and then transferred to a tissue culture room at 22 °C under a 12 h light/12 h dark photoperiod. After 7 d, seedlings were potted in soil mix (1:1 vermiculite:humus) and placed in a climate chamber at 22 °C, 70% relative humidity, and 150 μmol m<sup>-2</sup> s<sup>-1</sup> with a 12 h light/12 h darkness photoperiod.

### Localization of *TaABC1*-green fluorescent protein (GFP) fusion protein

The coding sequence of *TaABC1* was amplified with two primers (5'-GGT CTC AAG CTT ATG CCG CTG CCG CTG G-3'; *BsaI*

site in bold and *HindIII* site underlined) and (5'-GGT CTC CTC GAG AAA CAA CCT TCT AAG GAA ACT TCT-3'; *BsaI* site in bold and *BamHI* site underlined). The PCR product was subcloned into the pJIT163 vector to generate *pJIT163-TaABC1-GFP* containing a *TaABC1-GFP* fusion construct under control of the cauliflower mosaic virus (CaMV) 35S promoter. The construct was further confirmed by sequencing and used for transformation of onion (*Allium cepa*) epidermal cells by biolistic bombardment with a GeneGun (Bio-Rad Helios™) according to the instruction manual (helium pressure, 150–300 psi). The transformed cells were incubated in MS medium at 28 °C for 36–48 h and then observed with a laser scanning confocal microscope (Leika TCS-NT).

### Transformation of *TaABC1* in *Arabidopsis*

To produce *35S-TaABC1* plants, a 1444 bp fragment containing the coding sequence of *TaABC1* was amplified using two primers (5'-GGT ACC AGG CAG GGG GGC ATC-3'; the *KpnI* site underlined) and (5'-GGA TCC AAA CAA CCT TCT AAG GAA ACT TCT-3'; the *BamHI* site underlined). The PCR product was subcloned into the vector pPZP211 (Hajdukiewicz *et al.*, 1994), in which transgene expression is under control of the CaMV 35S promoter. Transformation of *Arabidopsis* was performed by the floral dip method (Clough and Bent, 1998) using *Agrobacterium tumefaciens* strain GV3101. Phenotypic analyses were performed on T<sub>3</sub> or T<sub>4</sub> homozygous lines.

### Drought, salt, and cold tolerance assays in transgenic *Arabidopsis*

Drought tolerance assays were performed on seedlings. *35S-TaABC1*, wild-type, and vector control seeds were germinated on MS medium. One-week-old seedlings were planted in identical pots containing mixed soil (1:1 vermiculite:humus) and well watered. The seedlings were cultured in a greenhouse (22 °C, 70% humidity, 150 μmol m<sup>-2</sup> s<sup>-1</sup>, 12 h light/12 h dark cycle) without watering.

Salt tolerance assays were conducted at the seedling stage on plates and in soil. For the salt tolerance assay on plates, wild-type, *35S-TaABC1*, and vector control seeds were germinated on MS medium. Five days after germination, seedlings from each line were carefully transferred to new MS media supplemented with different concentrations of NaCl. After 10 d growth in the treatment media, plants with no green or dead cotyledons were scored. *Arabidopsis* seedlings were cultured as described above for the salt tolerance assay in soil. Water was withheld for 4 weeks and plants were then well irrigated with NaCl solution (350 mM) applied at the bottom of the pots. When the soil was completely saturated with salt solution, free NaCl solution was removed and the plants were cultured under normal conditions. Survival rates were recorded 2 weeks later.

Cold tolerance assays were carried out on seedlings. Normally cultured *Arabidopsis* seedlings (4 weeks old) were stressed in a -8 °C freezer for 2 h, and subsequently cultured under normal growing conditions. Survival rates were scored after 6 d. All abiotic stress tolerance experiments were carried out in triplicate.

### Cell membrane stability

Plant cell membrane stability indices (MSIs) were determined with a conductivity meter (DDS-1, YSI); MSI (%)=(1-initial electrical conductivity/electrical conductivity after boiling)×100. Twenty 8-day-old seedlings (grown on MS medium, 0.8% agar) were transferred to a horizontal screen; seedling roots were completely submerged in NaCl solution (200 mM). When signs of stress began to appear on the control plants, seedlings were removed and immediately thoroughly rinsed with double-distilled water (ddH<sub>2</sub>O) prior to immersion in 20 ml of ddH<sub>2</sub>O at room temperature. After 2 h the initial conductivities of the solutions were recorded. The samples were then boiled for 30 min, cooled to room temperature, and the final conductivities were measured.

#### Water loss determination

Water loss was measured using 10 plants each of transgenic and control plants. Four-week-old plants were detached from roots and weighed immediately [fresh weight (FW)], and then the plants were left on the laboratory bench (humidity, 45–50%, 20–22 °C) and weighed at designated time intervals. The proportions of FW loss were calculated relative to the initial plant weights. The plants were finally dried for 24 h at 80 °C to a constant dry weight (DW). Relative water contents (RWCs) were measured according to the formula:  $RWC (\%) = (desiccated\ weight - DW) / (FW - DW)$ .

#### Osmotic potential (OP)

OP was measured with a Micro-Osmometer (Fiske® Model 210, Fiske® Associates). Measurements were taken in the freezing point mode at room temperature. Four 4-week-old plants of each line were collected as a sample, which was finely ground using a mortar and pestle before being transferred to a microcentrifuge tube. The supernatant sap was obtained after centrifuging at 12 000 rpm for 10 min at room temperature. Tests were done in triplicate.

#### Photochemistry efficiency assays

Photochemistry efficiency was measured with a portable modulated chlorophyll fluorometer (OS-30p, Opti-Sciences, USA). After fully expanded leaves were allowed to dark-adapt for 60 min, the maximum photochemistry efficiency of photosystem II (PSII) ( $F_v/F_m$ ) measurements were taken to assess changes in the primary photochemical reactions of photosynthetic potential at an early stage of drought stress. Three measurements were made for each plant, and 20 plants were used for transgenic and control lines.

#### Chlorophyll (Chl) content assay

Leaf Chl content measurements were performed as described by Hiscox and Israelstam (1979); that is, by measuring absorbance at 663 nm, and 645 nm of Chl extracted in dimethylsulphoxide (DMSO) using a spectrophotometer (SmartSpec™ 3000, Bio-Rad, USA). Transgenic and control plants were treated with 350 mM NaCl and measurements were made 24 h before and after the treatment.

#### Detection of reactive oxygen species (ROS)

For superoxide detection, detached fully expanded rosette leaves from 4-week-old transgenic and wild-type plants were harvested. Some of these leaves were incubated with 100 mM NaCl for 0.5/1.5 h, and others were incubated with ddH<sub>2</sub>O. These samples were vacuum-infiltrated with 0.1 mg ml<sup>-1</sup> nitroblue tetrazolium (NBT) in 25 mM HEPES buffer, pH 7.6. Samples were incubated at room temperature in the dark for 2 h. To remove Chl, stained samples were transferred to 80% ethanol and incubated at 70 °C for 10 min. Twenty leaves of each line were treated and measured for each treatment.

#### Gene expression analysis

Twelve-day-old seedlings grown on MS medium were treated, or not treated, with polyethylene glycol (PEG)-6000 (25.4%, -1.4 MPa). Total RNA was extracted using Trizol reagent and treated with RNase-free DNase. For real-time quantitative PCR, 2 µg of total RNA was used for retrotranscription with a SuperScript First-Strand Synthesis System kit (Invitrogen). Quantitative expression assays were performed with the SYBR Green Master Mix kit and an ABI 7300 sequence detection system according to the manufacturer's protocol (Applied Biosystem). RT-PCR conditions were as follows: 5 min at 95 °C, and 40 cycles of 15 s at 95 °C, 30 s at 60 °C, 31 s at 72 °C. Samples were run in triplicate on each 96-well plate and independent PCRs were repeated at least three times. The relative quantification method ( $\Delta\Delta CT$ ) was used to evaluate quantitative variation between replicates (Zhang *et al.*, 2008), and the *Actin* gene

was used as an internal control to normalize all data. The primer pairs used for real-time PCR are listed in Supplementary Table S1 available at *JXB* online.

## Results

### Sequence analysis, phylogenetic tree, and subcellular localization of TaABC1

Using the Conserved Domain Database search engine at the National Center for Biotechnology Information (Marchler-Bauer *et al.*, 2009), a conserved region of ~120 amino acids that is characteristic of the so-called ABC1 protein family (Fig. 1A) was detected in the putative TaABC1 protein sequence (HM773264). In addition, an aminoglycoside phosphotransferase choline kinase (APH\_ChoK) domain was also detected within the putative protein sequence of TaABC1 (Fig. 1A).

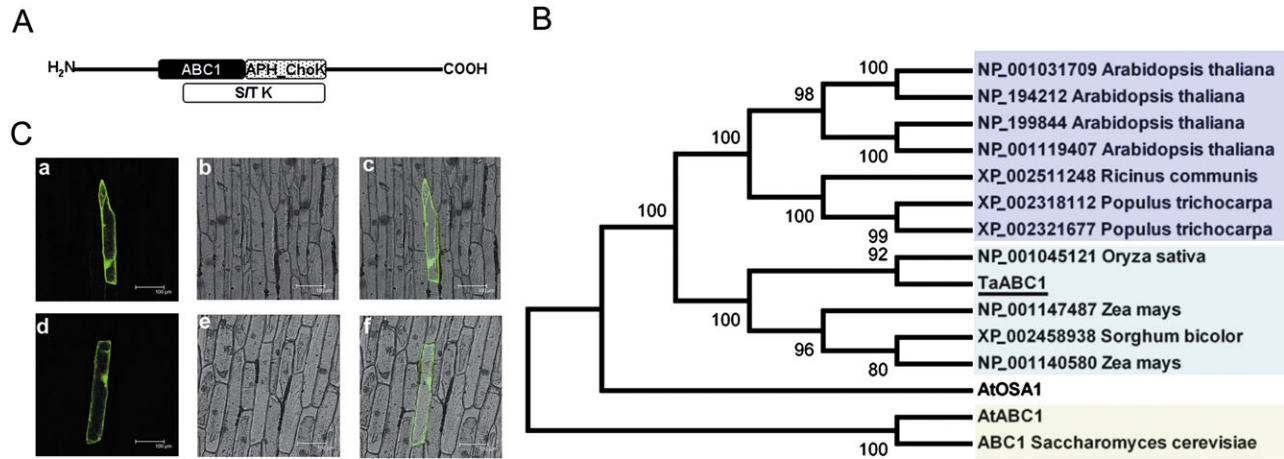
A phylogenetic tree was constructed based on the putative amino acid sequences of TaABC1, some plant ABC1 proteins, and yeast ABC1 protein (Fig. 1B). TaABC1 was clustered in the same monophyletic group as putative ABC1 proteins from rice, sorghum, and maize. In addition, TaABC1 protein showed >59.6% identity with putative ABC1 proteins from *Ricinuss communis*, *Populus tichocarpa*, and *A. thaliana*, but <30% identity with AtOSA1, AtABC1, and yeast ABC1. Furthermore, the results indicated that AtOSA1 protein did not group with AtABC1 and yeast ABC1 protein, but rather it formed a separate branch.

To examine its subcellular localization, TaABC1 was fused in-frame to the GFP gene under control of the CaMV 35S promoter. The constructs TaABC1-GFP and GFP were introduced into onion epidermal cells by particle bombardment. As shown in Fig. 1C, the TaABC1-GFP was localized to the cell membrane, cytoplasm, and nucleus.

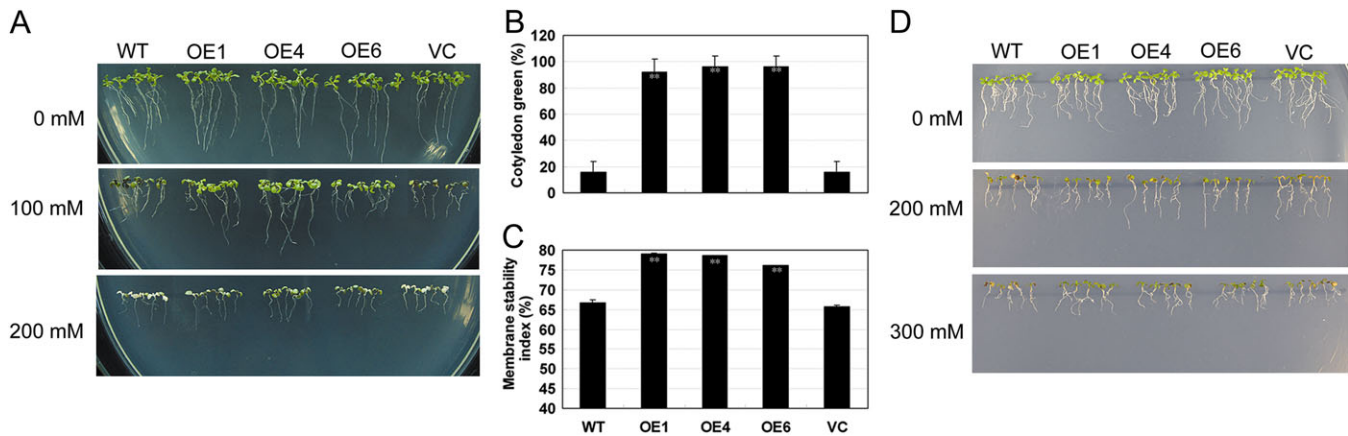
### Salt and osmotic responses of TaABC1-overexpressing Arabidopsis plants

Six T<sub>4</sub> homozygous transgenic lines were randomly selected for detection of gene expression. Further phenotypic analyses were performed on three lines (OE1, OE4, and OE6) which possess a higher expression level of *TaABC1* (Supplementary Fig. S1 at *JXB* online).

Salts inhibit seedling growth in a concentration-dependent manner (Xiong *et al.*, 2002). Previous studies showed that *TaABC1* was a salt-induced gene (Wang *et al.*, 2007), but the role of *TaABC1* in response to salt was not clear. The 35S-*TaABC1* *Arabidopsis* plants and the two controls were grown as described in the Materials and methods. Differences were observed at the young seedling stage. Cotyledon greening and expansion, as well as root growth of the control plants, were inhibited at the seedling stage on plates containing 100 mM NaCl (Fig. 2A). When the NaCl concentration was increased to 200 mM, the growth of control plants was strongly inhibited and <20% of seedlings had small green cotyledons, whereas *TaABC1* transgenic seedlings were still green (Fig. 2B). In the salinity stress tests, a significant difference was observed in terms of cell membrane stability between 8-day-old *TaABC1* transgenic



**Fig. 1.** Structure, localization, and homological analysis of TaABC1. (A) Schematic illustration of the TaABC1 protein structure. Identified domains are depicted as follows: black box, ABC1; dotted box, aminoglycoside phosphotransferase, choline kinase (APH\_Chok) domain; white box, Ser/Thr protein kinase (S/T K). (B) Phylogenetic tree of ABC1 proteins. The tree was constructed with the MEGA 4.1 program; bootstrap values are in percentages. (C) Subcellular localization of TaABC1 protein by GFP fusion expression in onion epidermal cells. The GFP control or TaABC1-GFP fusion was driven by the CaMV 35S promoter. Cells were bombarded with DNA-coated gold particles and analysed by fluorescence microscopy. Photographs were taken after 16 h of incubation. Panels from left to right: images of GFP control (a) or TaABC1-GFP (d) in dark field, in bright field (b and e), and the combined images (c and f).



**Fig. 2.** Salt and osmotic tolerance of *35S-TaABC1*, wild-type (WT), and vector control (VC) plants. (A) NaCl effects on newly germinated seedling growth. *35S-TaABC1* (lines OE1, OE4, and OE6), WT, and VC seeds were planted on MS medium for germination. Five days after germination, seedlings from each line were carefully transferred to a new MS medium containing 100 mM and 200 mM NaCl. Photographs of representative seedlings of *35S-TaABC1*, WT, and VC plants were taken after 10 d of growth in the treatment medium. (B) NaCl dose-response analysis of post-germinative growth (green cotyledons). Results were scored at 10 d on MS medium containing 200 mM NaCl. Values are the mean  $\pm$ SD (triplicate measurements;  $n=15$ ). (C) Cell membrane stability measurement of *35S-TaABC1*, WT, and VC plants under 200 mM NaCl treatment. Values are the mean  $\pm$ SD (triplicate measurements;  $n=20$ ). (D) Mannitol effects on newly germinated seedling growth. *35S-TaABC1*, WT, and VC seeds were germinated for 5 d on MS medium and then transferred to a new MS medium containing 200 mM and 300 mM mannitol for an additional 10 d of growth. Representative seedlings are shown.

and control plants. After the 200 mM NaCl treatment was applied for 5 h, the control plants began to show symptoms of salt stress, whereas no signs of stress were shown on *TaABC1* plants. MSI determinations showed that the cell membrane stability of *TaABC1* plants was 12–16% higher than that of the control plants (Fig. 2C), and the difference was highly significant ( $P < 0.01$ ).

To determine further if *TaABC1* was involved in the response to salt stress or general osmotic effect stress, 5-day-old seedlings grown on MS medium were transferred to MS medium with 200 mM and 300 mM mannitol (an osmotic agent) for 10 d. The growth of control plants on plates containing different concentrations of mannitol was slightly inhibited compared with *35S-TaABC1* plants (Fig. 2D),

suggesting that TaABC1 is involved in a general osmotic response to salt.

#### *Drought response of TaABC1-overexpressing Arabidopsis plants*

To characterize the performance of *35S-TaABC1* plants under drought stress, 7-day-old *35S-TaABC1*, wild-type, and vector control plants were grown in soil for an additional 4 weeks (initial stage of drought stress). Thereafter, plants were not watered for 15 d (late stage of drought stress) to induce drought stress. The plants were then rewatered after 3 d (Fig. 3A). Before rewatering, the wilting levels of wild-type plants and vector control plants were more obvious than those of the *35S-TaABC1* plants. After rewatering, 49–80% of *35S-TaABC1* plants survived, whereas the corresponding survival rates were 25% for wild-type plants and 11% for vector control plants (Fig. 3B).

To assess the water retention ability of *35S-TaABC1* plants, FW losses in detached rosette leaves were measured. The FW loss of detached rosette leaves in *35S-TaABC1* transgenic plants was <38%, as opposed to 49% and 50% for wild-type and vector control plants, respectively (Fig. 3C). The final RWCs of the *35S-TaABC1* plants were significantly higher than those of the control plants (Fig. 3D). This suggests that the *35S-TaABC1* plants possess higher water retention ability than wild-type and vector control plants.

An OP assay was undertaken to assess the osmotic adjustment ability of *35S-TaABC1* plants. The OP of *35S-TaABC1* transgenic plants was significantly higher than those of control plants, and there was no significant difference between the two controls (Fig. 3E). Overexpression of *TaABC1* apparently led to enhanced OP.

To evaluate further the photosynthetic activity of *35S-TaABC1* plants, 1-week-old *35S-TaABC1*, wild-type, and vector control plants were transplanted to soil and grown for an additional 22 d and 28 d (approximately normal conditions and moderate drought stress conditions, respectively), and the maximum photochemistry efficiencies of PSII ( $F_v/F_m$ ) were measured. Under normal conditions, there were no evident differences between *35S-TaABC1* plants and the controls in  $F_v/F_m$  ratio. Under moderate drought stress conditions, Chl fluorescence values showed that the  $F_v/F_m$  ratios of the *35S-TaABC1* plants were significantly higher than those of the control plants ( $P < 0.01$ ) (Fig. 3F).

#### *Salt response of TaABC1-overexpressing Arabidopsis plants*

To examine further salt stress responses of *TaABC1* transgenics, *35S-TaABC1* and control plants were grown in potted soil for 4 weeks, and then soaked with 350 mM NaCl solution. About 3 d after NaCl treatment, the control plants withered, whereas the leaves of transgenic plants were still green. Ten days later, signs of salt stress were

evident; *35S-TaABC1* plants were much less affected than the control plants (Fig. 4A). The survival rates of *TaABC1* transgenics were much higher than those of the controls (Fig. 4B).

The Chl contents of *35S-TaABC1* and control plants were measured before and after 350 mM NaCl treatment. There were no significant differences in Chl content between *35S-TaABC1* and control plants under normal conditions but, 18 h after 350 mM NaCl treatment, Chl contents of all plants were reduced and leaf Chl contents of the control plants decreased more rapidly than those of the *35S-TaABC1* plants ( $P < 0.01$ ) (Fig. 4C). These results indicated that the retention of stay-green features in *35S-TaABC1* plants was superior to that of the control plants.

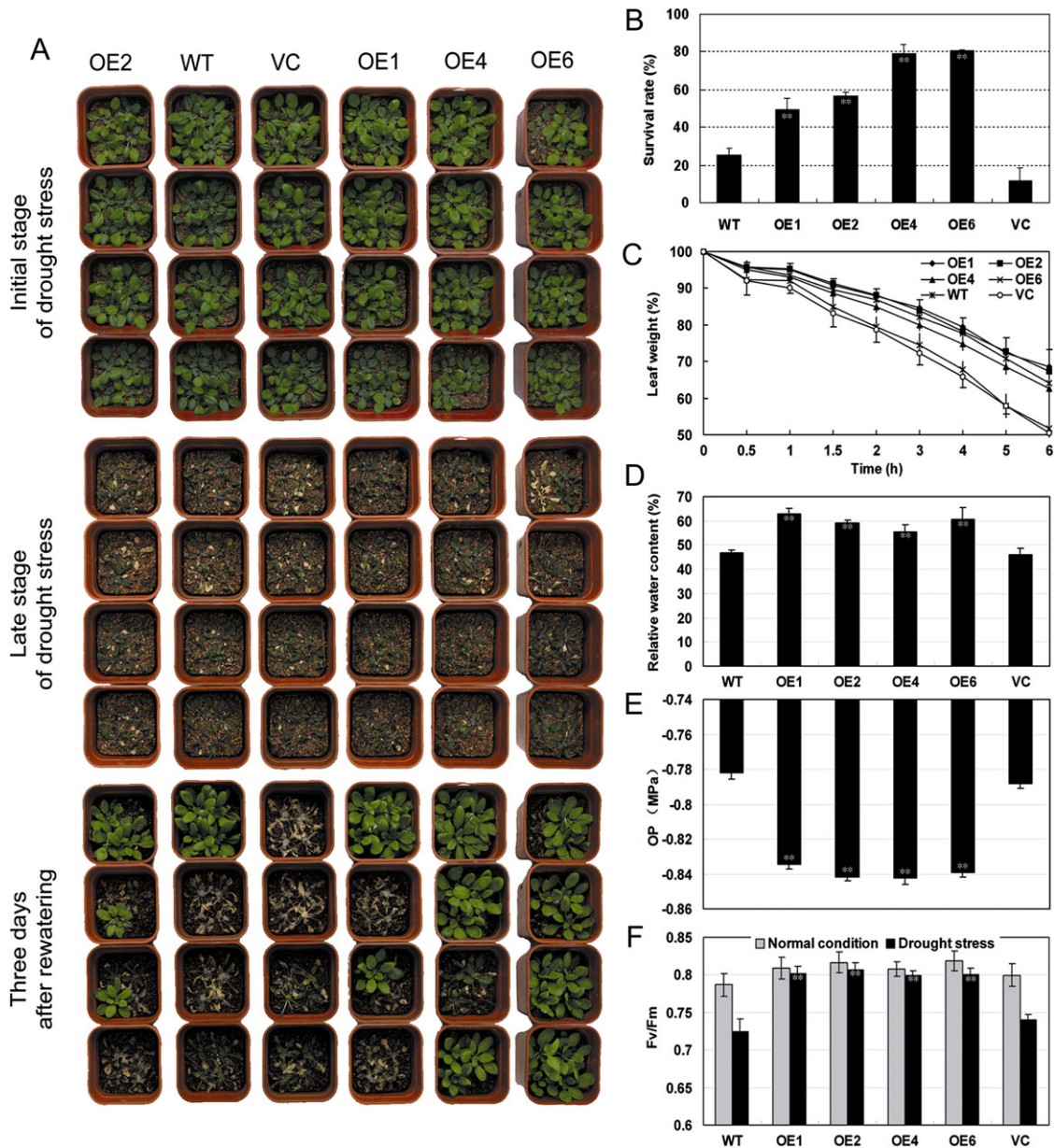
Salt stress also leads to an accumulation of high levels of ROS (Van Camp *et al.*, 1996; Borsani *et al.*, 2001). The presence of ROS was further examined with NBT staining for superoxide in the *35S-TaABC1* and control plants with or without salt stress. Under non-salt stress conditions, *35S-TaABC1* and control leaves showed slight NBT staining, indicative of low levels of superoxide (Fig. 4D). However, under stress conditions, the staining levels of control leaves were much higher than in *35S-TaABC1* leaves (Fig. 4D) showing that *35S-TaABC1* plants scavenged superoxide more effectively than the controls.

#### *Cold response of TaABC1-overexpressing Arabidopsis plants*

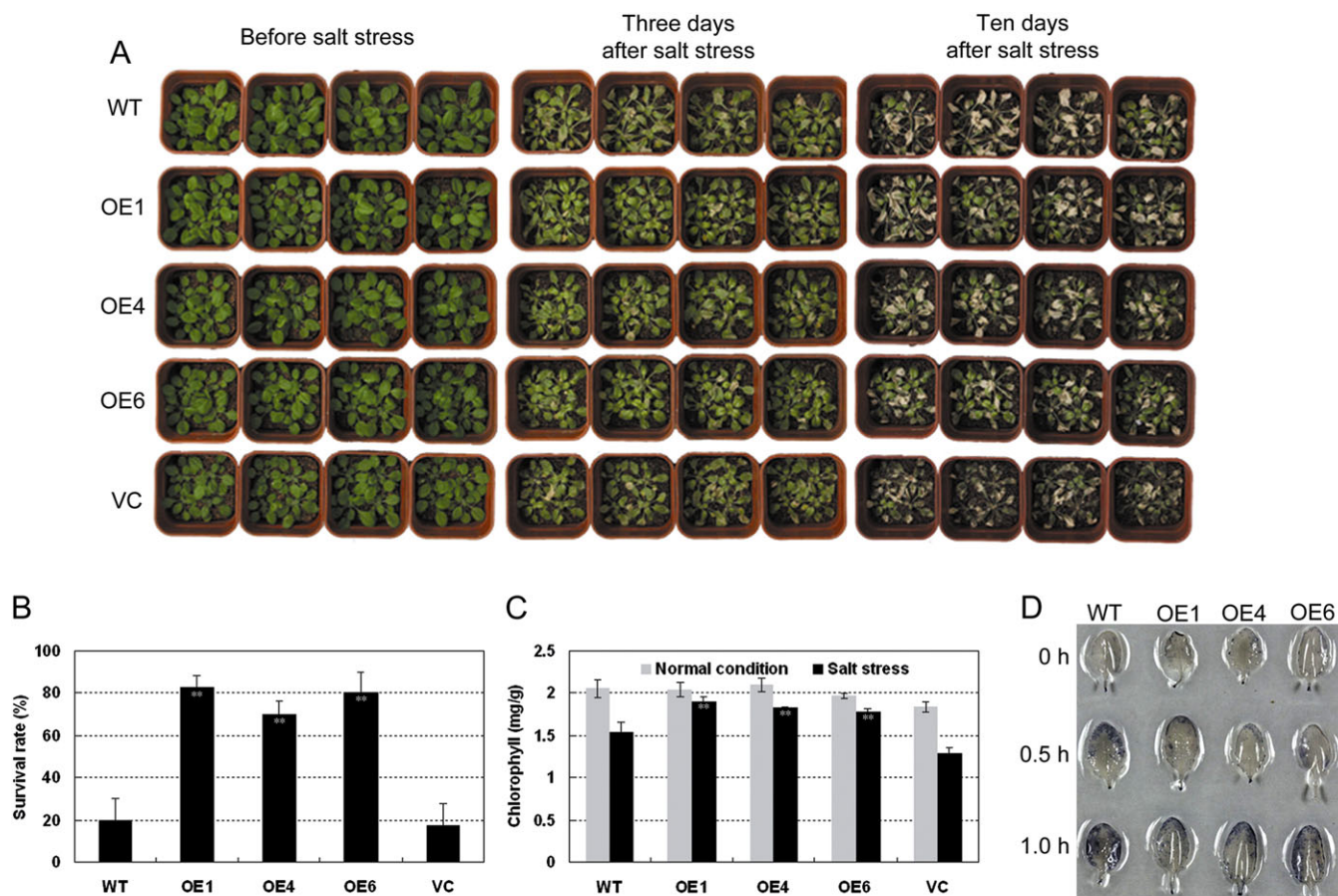
Because *TaABC1* is also induced by cold (Wang *et al.*, 2007), it was expected that *35S-TaABC1* plants would have altered responses to cold. Four-week-old *35S-TaABC1* and control plants were exposed to  $-8\text{ }^\circ\text{C}$  for 1.5 h. After 1 of recovery under normal conditions, most *35S-TaABC1* plants were phenotypically green, whereas most of the control plants were dead (Fig. 5A); 6–16% of the control plants and 58–69% of the *35S-TaABC1* plants grew normally after recovery (Fig. 5B). These data suggest that *TaABC1*-overexpressing plants have increased tolerance to freezing stress.

#### *Expression of stress-responsive genes in TaABC1-overexpressing Arabidopsis plants*

To elucidate the molecular mechanism of *TaABC1* in osmotic response, the expression of stress-responsive genes identified in different regulated pathways was monitored by real-time PCR in transgenic *Arabidopsis*. Under normal conditions, the expression of *DREB2A*, *RD29A*, *ABF3*, *KIN1*, *DREB1B/CBF1*, *LEA*, and *P5CS* in *TaABC1*-overexpressing plants was substantially increased compared with wild-type plants, whereas there was no significant induction of expression of *DREB1A/CBF3* in either transgenic or wild-type plants (Fig. 6). However, with PEG treatment for 2 h, the expressions of the tested marker genes, including *DREB1A/CBF3*, *DREB2A*, *RD29A*, *ABF3*, *KIN1*, *DREB1B/CBF1*, *LEA*, and *P5CS*, was up-regulated in both wild-type and



**Fig. 3.** Responses to drought of *35S-TaABC1*, wild-type (WT), and vector control (VC) plants. (A) Drought tolerance assays of *35S-TaABC1* (lines OE1, OE2, OE4, and OE6), WT, and VC plants. One-week-old *35S-TaABC1*, WT, and VC plants, were transplanted to soil for an additional 4 weeks (initial stage of drought stress). Thereafter, plants continued to be not watered for 15 d (late stage of drought stress) to induce drought stress. Photographs of representative seedlings of *35S-TaABC1*, WT, and VC plants were taken at the initial stage of drought stress, late stage of drought stress, and 3 d after rewatering. (B) Quantitative analysis of survival of *35S-TaABC1* (lines OE1, OE2, OE4, and OE6), WT, and VC plants 3 d after rewatering. Values are the mean  $\pm$ SD (triplicate measurements;  $n=20$  plants). (C) Water loss rates of *35S-TaABC1* (lines OE1, OE2, OE4, and OE6), WT, and VC plants. Detached leaves from 4-week-old plants grown on soil were left on the laboratory bench (humidity, 45–50%, 20–22 °C) and weighed at various time points after detachment. The proportions of fresh weight loss were calculated relative to the initial plant weights. Values are mean  $\pm$ SD ( $n=10$  plants). (D) Relative water contents of *35S-TaABC1* (lines OE1, OE2, OE4, and OE6), WT, and VC plants. Detached leaves from 4-week-old plants grown on soil were left on the laboratory bench (humidity, 45–50%, 20–22 °C) and weighed at various time points after detachment. The plants were finally dried for 24 h at 80°C to a constant dry weight (DW). Relative water contents were measured according to the formula:  $RWC (\%) = (\text{desiccated weight} - DW) / (FW - DW)$ . Values are mean  $\pm$ SD ( $n=10$  plants). (E) Osmotic potential (OP) measurement of *35S-TaABC1* (lines OE1, OE2, OE4, and OE6), WT, and VC plants. Values are the mean  $\pm$ SD (triplicate measurements;  $n=20$  plants). (F) Photochemical efficiency measurement of *35S-TaABC1* (lines OE1, OE2, OE4, and OE6), WT, and VC plants. One-week-old *35S-TaABC1*, WT, and VC plants, were transplanted to soil for an additional 22 d and 28 d (normal conditions and moderate drought stress conditions) prior to measuring photochemical efficiencies ( $F_v/F_m$ ). Values are the mean  $\pm$ SD (triplicate measurements;  $n=20$  plants).



**Fig. 4.** Responses to salt of *35S-TaABC1*, wild-type (WT), and vector control (VC) plants. (A) Four-week-old *35S-TaABC1* (lines OE1, OE4, and OE6), WT, and VC plants were irrigated with NaCl solution (350 mM); free NaCl solution was removed and the plants were cultured under normal conditions. Photographs of representative seedlings were taken 3 d and 10 d after treatment. (B) Quantitative analysis of *35S-TaABC1* (lines OE1, OE4, and OE6), WT, and VC plant survival 10 d after 350 mM NaCl treatment. Values are the mean  $\pm$ SD (triplicate measurements;  $n=20$  plants). (C) Chl contents in leaves before or after 350 mM NaCl treatment for 18 h. Values are the mean  $\pm$ SD of triplicate measurements. (D) ROS production in leaves of *35S-TaABC1* and WT plants. Four-week-old detached leaves were stained with nitroblue tetrazolium (NBT). The photographs are representative leaves from three independent experiments.

transgenic plants. Thus, overexpression of *TaABC1* increases expression of stress-responsive genes in *Arabidopsis*.

## Discussion

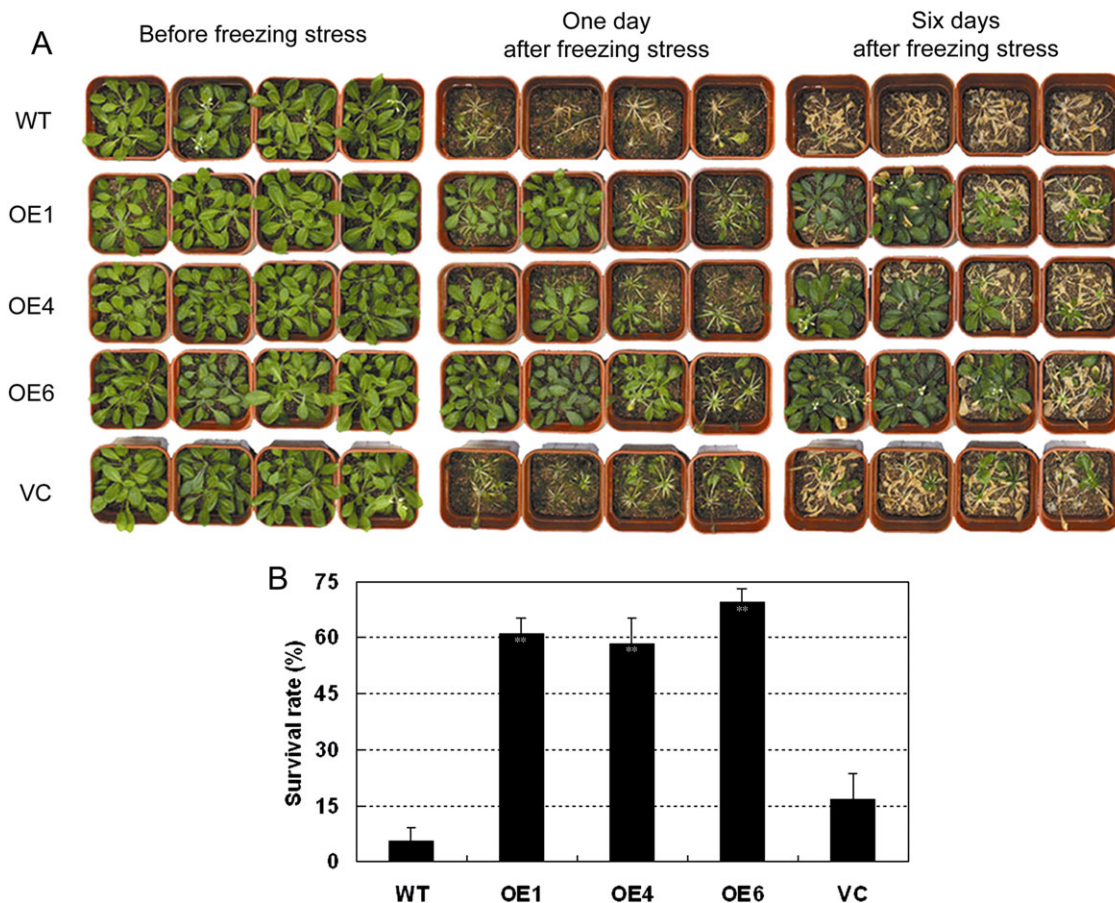
Plants have acquired an ability to survive stress conditions by developing highly organized signalling networks, in which protein kinase phosphorylation is one of the central signalling events that occur in response to environmental stress (Ichimura *et al.*, 2000). The effects of overexpression of *TaABC1*, a wheat ABC1 protein kinase, in *Arabidopsis* were investigated and it was found that it induced various stress-responsive genes involved in stress signalling pathways. The transgenic plants had increased tolerance to drought, salt, and cold stress.

*TaABC1*-encoded protein kinase localizes to the cell membrane, cytoplasm, and nucleus

Phylogenetic analysis revealed that *AtOSAI* does not group with *AtABC1* (Fig. 1B), in agreement with Jasinski *et al.* (2008).

The present studies also showed that *TaABC1* does not cluster with *AtABC1* or *AtOSAI*; rather, it clustered with putative ABC1 proteins from rice, sorghum, and maize. *TaABC1* protein showed >59.6% identities with putative ABC1 proteins from *R. communis*, *P. tichocarpa*, and *A. thaliana*. It is therefore speculated that although ABC1 family proteins possess the conserved ABC1 domain, they also contain other kinase domains, partially explaining why they share high identity levels, but cluster into different groups.

Only two ABC1 genes are characterized amongst 17 genes containing typical ABC1 motifs in *Arabidopsis* (Cardazzo *et al.*, 1998; Jasinski *et al.*, 2008). The first representative ABC1 in plants (*AtABC1*, At4g01660) is a structural and functional homologue of yeast ABC1, and it allows partial restoration of the complex III activity of a yeast *abc1* mutant, suggesting subcellular localization of *AtABC1* in mitochondria (Bousquet *et al.*, 1991; Cardazzo *et al.*, 1998). However, *AtOSAI*, another ABC1 gene in *Arabidopsis*, is located in chloroplasts as a further factor playing a role in



**Fig. 5.** Responses of *35S-TaABC1*, wild-type (WT), and vector control (VC) plants to cold. (A) Four-week-old *35S-TaABC1* (lines OE1, OE4, and OE6), WT, and VC plants were cold stressed at  $-8^{\circ}\text{C}$  for 2 h and then transferred to normal conditions for recovery. Photographs of representative seedlings of *35S-TaABC1*, WT, and VC plants were taken after 1 d and 6 d of recovery. (B) Survival rates were determined as the number of visibly green plants after rehydration. Values are the mean  $\pm$ SD (triplicate measurements;  $n=15$  plants).

the balance of oxidative stress (Jasinski *et al.*, 2008). TaABC1 protein was present in the cell membrane, cytoplasm, and nucleus (Fig. 1C). These localizations are different from those reported for AtABC1 and AtOSA1. This further confirms the view that ABC1 family proteins contain the conserved ABC1 domain, but have different kinase domains that lead to different localizations of ABC1 proteins and presumably the diverse functions they perform. Protein phosphorylation, catalysed by protein kinases, is one of the major post-translational modifications involved in the signal transduction pathway. It is generally acknowledged that signal transduction occurs at the plasma membrane level, in the cytosol, and at the transcriptional level (Hardie, 1999; Olsen *et al.*, 2006; Afzal *et al.*, 2008; Colcombet and Hirt, 2008; de la Fuente van Bentem and Hirt, 2009). However, strictly nucleus-localized and nucleus-cytosol-localized protein kinases are attracting increasing attention in signalling networks (Pandey *et al.*, 2002; Cheong *et al.*, 2003; Dammann *et al.*, 2003; Riera *et al.*, 2004; Choi *et al.*, 2005; Raichaudhuri *et al.*, 2006; Vert and Chory, 2006; Salinas *et al.*, 2006; Takahashi *et al.*, 2007; Yoo *et al.*, 2008). Therefore, some researchers assume that nucleocytoplasmic trafficking machinery probably controls cytosolic translocation

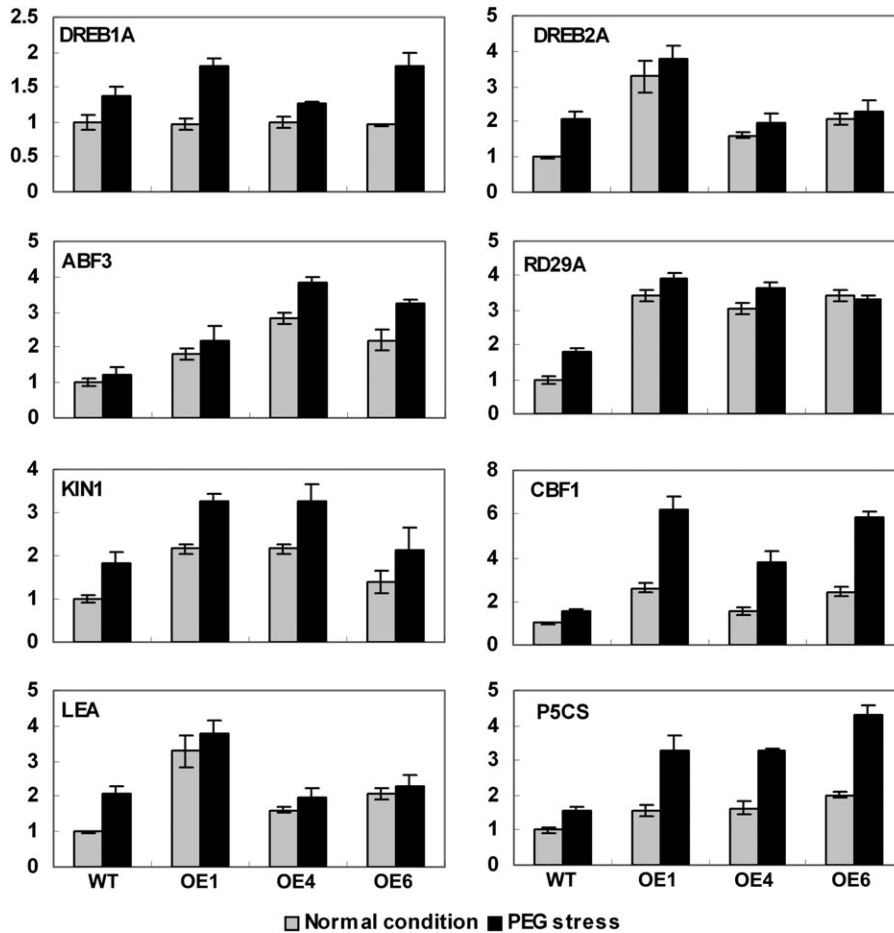
of an activated form, or activation in the nucleus through promoting interactions with their substrates under specific cellular conditions (Dahan *et al.*, 2010).

#### *Overexpression of TaABC1 enhances tolerance to abiotic stress in Arabidopsis*

Dehydration, salinity and low temperature stresses in plants lead to membrane disorganization, inhibition of photosynthesis, and generation of ROS (Hasegawa *et al.*, 2000).

Maintenance of membrane integrity and stability is thought to be a major component of environmental stress tolerance (Levitt, 1980). Cell membrane stability is positively correlated with various physiological and biochemical parameters conditioning responses to environmental stresses such as changes in OP, leaf rolling index, and/or leaf RWC (Munns, 2002). The cell MSI under environmental stress can be easily estimated by measurements of electrolyte leakage from cells. In this study, the MSI of *35S-TaABC1* plants under osmotic stress was higher than that of control plants (Fig. 2C). Because cell membrane stability is positively related to OP and RWC, the RWCs and OP of detached leaves were further tested. The RWCs of *35S-TaABC1*





**Fig. 6.** Expression of stress-responsive genes in *35S-TaABC1* and wild-type plants induced by PEG (−1.4 MPa) using real-time PCR. Total RNA was extracted from 2-week-old plants grown under normal or PEG treatment for 2 h. Transcript levels were measured by real-time RT-PCR of *DREB1A*, *DREB2A*, *RD29A*, *ABF3*, *KIN1*, *CBF1*, *LEA*, and *P5CS* under normal conditions (grey bars) or PEG treatment for 2 h (black bars). *Actin* was used as an internal control. Data represent the mean ±SD of three replicates.

plants were higher than those of the control plants (Fig. 3D). A similar result was obtained for OP. The OP of *35S-TaABC1* plants was significantly higher than that of the control plants under well-watered conditions (Fig. 3E). These results indicated that *TaABC1*-overexpressing transgenic *Arabidopsis* had higher water retention and osmotic adjustment abilities, and thus increased tolerance to abiotic stresses.

Abiotic stresses provoke oxidative damage to photosynthetic proteins and pigments. The photochemical efficiency of PSII ( $F_v/F_m$ ) and Chl content have been used as physiological senescence markers (Krause and Weis, 1991; Franke and Schreiber, 2007; Lim *et al.*, 2007; Schippers *et al.*, 2008; Osakabe *et al.*, 2010). The effect of drought and salt stress on the levels of these senescence markers in *35S-TaABC1* and control plants was investigated. The drought and salt treatments caused severe damage to photochemical efficiency and Chl contents of wild-type plants (Figs 3F, 4C). ROS homeostasis is maintained by production of ROS, scavenging enzymes and non-enzymatic antioxidants (Mustilli *et al.*, 2002; Mittler *et al.*, 2004; Davletova *et al.*, 2005). Superoxides in *35S-TaABC1* and wild-type plants were measured by NBT staining under salt stress conditions. It was

found that transgenic plants overexpressing *TaABC1* had enhanced tolerance to oxidative stress compared with wild-type plants (Fig. 4D). These results confirmed that overexpression of *TaABC1* reduced the levels of damage to photosynthetic proteins and pigments, thereby increasing stress tolerance levels in transgenic *Arabidopsis*.

Generally, drought tolerance accompanies hypersensitivity to ABA treatments during seed germination and early seedling development (Hu *et al.*, 2006; Ko *et al.*, 2006; Zhang *et al.*, 2007; Osakabe *et al.*, 2010). However, in the system used here, enhanced tolerance to stress is not accompanied by sensitivity of seed germination and early seedling development to ABA in overexpressed *TaABC1* transgenic plants (data not shown). The understanding is that ABA dependence might not be the major mode by which *TaABC1* is involved in drought, salt, and cold stress signalling pathways. It is possible that expression of related genes in other signal pathways increases the OP and reduces the levels of damage to photosynthetic proteins and pigments to enhance multistress tolerance. Under stress conditions, enhancement of OP leads to a decrease of water loss, an increase in RWC, maintenance of regular cell turgor, and avoidance of damage to cell membranes. The

higher OP probably also prevents entry of harmful ions, and relieves ion damage to cell membranes; and, moreover, it commonly lowers freezing points in plant cells. In addition, the levels of damage to photosynthetic proteins and pigments decline, thereby maintaining life under stress conditions. Finally, overexpression of *TaABC1* conferred tolerance to drought, salt, and cold.

#### *TaABC1 enhances multiple stress-responsive genes*

Overexpression of genes such as *DREB1A/CBF3*, *DREB2A*, *RD29A*, *ABF3*, *DREB1B/CBF1*, and *P5CS* conferred stress tolerance in transgenic plants (Yamaguchi-Shinozaki and Shinozaki, 1994; Kishor *et al.*, 1995; Jaglo-Ottosen *et al.*, 1998; Liu *et al.*, 1998; Kasuga *et al.*, 1999; Kang *et al.*, 2002; Dubouzet *et al.*, 2003; Sakuma *et al.*, 2006). To identify whether *TaABC1* affects the expression of stress-related genes, eight marker genes involved in the stress response were compared in *TaABC1*-overexpressing and wild-type plants. Overexpression of *DREB1/CBF* in transgenic plants increased tolerance to freezing, drought, and salt stresses (Liu *et al.*, 1998; Kasuga *et al.*, 1999; Dubouzet *et al.*, 2003). Ito *et al.* (2006) showed that overexpression of *OsDREB1* also improved drought and chilling tolerance in rice (Ito *et al.*, 2006). However, the results failed to show that *DREB1A/CBF3* underwent increased expression in *35S-TaABC1* plants under normal conditions (Fig. 6), suggesting that there may be other stress pathways involved in *TaABC1*-mediated stress tolerance.

The *RD29A* gene is a drought-, cold-, and ABA-inducible gene with a dehydration-responsive element (DRE) and an ABA-responsive element (ABRE) present in its promoter region (Shinozaki and Yamaguchi-Shinozaki, 1997). Overexpression of constitutively active *DREB2A* resulted in significant drought stress tolerance, but only slight freezing tolerance in transgenic *Arabidopsis* plants (Sakuma *et al.*, 2006). *ABF3*, *KINI1*, and *DREB1B/CBF1* are thought to be involved in different stress regulation pathways (Yamaguchi-Shinozaki and Shinozaki, 2006). The high transcript levels of *DREB2A*, *RD29A*, *ABF3*, *KINI1*, and *DREB1B/CBF1* in *35S-TaABC1* plants suggest that *TaABC1* may act upstream of these genes in stress tolerance and is therefore involved in a cross-talk between the complex networks of stress-responsive genes.

Late embryogenesis abundant (LEA) proteins and  $\Delta^1$ -pyrroline-5-carboxylate synthetase (P5CS) function in the protection of cells during osmotic stress (Taji *et al.*, 2002; Battaglia *et al.*, 2008). The present study showed that the expression levels of *LEA* and *P5CS* were up-regulated in *35S-TaABC1* plants under normal and stress conditions (Fig. 6). This is consistent with higher OP in *TaABC1* plants.

The expression of *DREB1A/CBF3* showed no clear changes in *35S-TaABC1* plants, but *DREB1B/CBF1*, *DREB2A*, and *ABF3* underwent increased expression under normal conditions. This demonstrated that *TaABC1* was possibly more dependent on the *DREB1B/CBF1*, *DREB2A*, and *ABF3* stress pathways. Moreover, it seems likely that

cross-talk among different stress signalling pathways occurs in *TaABC1*-induced stress responses.

Based on various analyses, it is speculated that the higher tolerance of *TaABC1* plants to drought, salt, and cold stresses may be due to the combined effects of up-regulated expression of stress-responsive genes, increased OP, and decreased damage to photosynthetic proteins and pigments.

## Supplementary data

Supplementary data are available at *JXB* online.

**Figure S1.** Expression levels of *TaABC1* in different transgenic *Arabidopsis* lines.

**Table S1.** Primer pairs used in quantitative real-time PCR.

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## References

- Afzal AJ, Wood AJ, Lightfoot DA.** 2008. Plant receptor-like serine threonine kinases: roles in signaling and plant defense. *Molecular Plant-Microbe Interactions* **21**, 507–517.
- Bartels D, Sunkar R.** 2005. Drought and salt tolerance in plants. *Critical Reviews in Plant Science* **24**, 23–58.
- Baticados W, Witola W, Inoue N, Kim J, Kuboki N, Xuan X, Yokoyama N, Sugimoto C.** 2005. Expression of a gene encoding *Trypanosoma congolense* putative Abc1 family protein is developmentally regulated. *Journal of Veterinary Medical Science* **67**, 157–164.
- Battaglia M, Olvera-Carrillo Y, Garcarrubio A, Campos F, Covarrubias AA.** 2008. The enigmatic LEA proteins and other hydrophilins. *Plant Physiology* **148**, 6–24.
- Borsani O, Valpuesta V, Botella MA.** 2001. Evidence for a role of salicylic acid in the oxidative damage generated by NaCl and osmotic stress in *Arabidopsis* seedlings. *Plant Physiology* **126**, 1024–1030.
- Bousquet I, Dujardin G, Slonimski P.** 1991. ABC1, a novel yeast nuclear gene, has a dual function in mitochondria: it suppresses a cytochrome b mRNA translation defect and is essential for the electron transfer in the bc 1 complex. *EMBO Journal* **10**, 2023–2031.
- Brasseur G, Tron G, Dujardin G, Slonimski PP, Brivet-Chevillotte P.** 1997. The nuclear ABC1 gene is essential for the correct conformation and functioning of the cytochrome bc1 complex and the neighbouring complexes II and IV in the mitochondrial respiratory chain. *European Journal of Biochemistry* **246**, 103–111.
- Cardazzo B, Hamel P, Sakamoto W, Wintz H, Dujardin G.** 1998. Isolation of an *Arabidopsis thaliana* cDNA by complementation of

a yeast abc1 deletion mutant deficient in complex III respiratory activity. *Gene* **221**, 117–125.

**Cheong YH, Moon BC, Kim JK, et al.** 2003. BWMK1, a rice mitogen-activated protein kinase, locates in the nucleus and mediates pathogenesis-related gene expression by activation of a transcription factor. *Plant Physiology* **132**, 1961–1972.

**Choi HI, Park HJ, Park JH, Kim S, Im MY, Seo HH, Kim YW, Hwang I, Kim SY.** 2005. Arabidopsis calcium-dependent protein kinase AtCPK32 interacts with ABF4, a transcriptional regulator of abscisic acid-responsive gene expression, and modulates its activity. *Plant Physiology* **139**, 1750–1761.

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

**Colcombet J, Hirt H.** 2008. Arabidopsis MAPKs: a complex signalling network involved in multiple biological processes. *Biochemical Journal* **413**, 217–226.

**Dahan J, Wendehenne D, Ranjeva R, Pugin A, Bourque S.** 2010. Nuclear protein kinases: still enigmatic components in plant cell signalling. *New Phytologist* **185**, 355–368.

**Dammann C, Ichida A, Hong B, Romanowsky SM, Hrabak EM, Harmon AC, Pickard BG, Harper JF.** 2003. Subcellular targeting of nine calcium-dependent protein kinase isoforms from *Arabidopsis*. *Plant Physiology* **132**, 1840–1848.

**Davletova S, Rizhsky L, Liang H, Shengqiang Z, Oliver D, Couto J, Shulaev V, Schlauch K, Mittler R.** 2005. Cytosolic ascorbate peroxidase 1 is a central component of the reactive oxygen gene network of *Arabidopsis*. *The Plant Cell* **17**, 268–281.

**de la Fuente van Bentem S, Hirt H.** 2009. Protein tyrosine phosphorylation in plants: more abundant than expected? *Trends in Plant Science* **14**, 71–76.

**Do T, Hsu A, Jonassen T, Lee P, Clarke C.** 2001. A defect in coenzyme Q biosynthesis is responsible for the respiratory deficiency in *Saccharomyces cerevisiae* abc1 mutants. *Journal of Biological Chemistry* **276**, 18161–18168.

**Dubouzet JG, Sakuma Y, Ito Y, Kasuga M, Dubouzet EG, Miura S, Seki M, Shinozaki K, Yamaguchi-Shinozaki K.** 2003. OsDREB genes in rice, *Oryza sativa* L., encode transcription activators that function in drought-, high-salt- and cold-responsive gene expression. *The Plant Journal* **33**, 751–763.

**Franke R, Schreiber L.** 2007. Suberin—a biopolyester forming apoplastic plant interfaces. *Current Opinion in Plant Biology* **10**, 252–259.

**Fujita M, Fujita Y, Noutoshi Y, Takahashi F, Narusaka Y, Yamaguchi-Shinozaki K, Shinozaki K.** 2006. Crosstalk between abiotic and biotic stress responses: a current view from the points of convergence in the stress signaling networks. *Current Opinion in Plant Biology* **9**, 436–442.

**Hajdukiewicz P, Svab Z, Maliga P.** 1994. The small, versatile pPZP family of *Agrobacterium* binary vectors for plant transformation. *Plant Molecular Biology* **25**, 989–994.

**Hardie DG.** 1999. Plant protein serine/threonine kinase: classification and functions. *Annual Review of Plant Physiology and Plant Molecular Biology* **50**, 97–131.

**Hasegawa PM, Bressan RA, Zhu JK, Bohnert HJ.** 2000. Plant cellular and molecular responses to high salinity. *Annual Review of Plant Physiology and Plant Molecular Biology* **51**, 463–499.

**Hiscox J, Israelstam G.** 1979. A method for the extraction of chlorophyll from leaf tissue without maceration. *Canadian Journal of Botany* **57**, 1332–1334.

**Hsieh E, Dinoso J, Clarke C.** 2004. A tRNATRP gene mediates the suppression of cbs2-223 previously attributed to ABC1/COQ8. *Biochemical and Biophysical Research Communications* **317**, 648–653.

**Hu H, Dai M, Yao J, Xiao B, Li X, Zhang Q, Xiong L.** 2006. Overexpressing a NAM, ATAF, and CUC (NAC) transcription factor enhances drought resistance and salt tolerance in rice. *Proceedings of the National Academy of Sciences, USA* **103**, 12987–12992.

**Ichimura K, Mizoguchi T, Yoshida R, Yuasa T, Shinozaki K.** 2000. Various abiotic stresses rapidly activate Arabidopsis MAP kinases ATMPK4 and ATMPK6. *The Plant Journal* **24**, 655–665.

**Iizumi M, Arakawa H, Mori T, Ando A, Nakamura Y.** 2002. Isolation of a novel gene, CAB1, encoding a mitochondrial protein that is highly homologous to yeast activity of bc1 complex. *Cancer Research* **62**, 1246–1250.

**Ingram J, Bartels D.** 1996. The molecular basis of dehydration tolerance in plants. *Annual Review of Plant Physiology and Plant Molecular Biology* **47**, 377–403.

**Ito Y, Katsura K, Maruyama K, Taji T, Kobayashi M, Seki M, Shinozaki K, Yamaguchi-Shinozaki K.** 2006. Functional analysis of rice DREB1/CBF-type transcription factors involved in cold-responsive gene expression in transgenic rice. *Plant and Cell Physiology* **47**, 141–153.

**Jaglo-Ottosen KR, Gilmour SJ, Zarka DG, Schabenberger O, Thomashow MF.** 1998. Arabidopsis CBF1 overexpression induces COR genes and enhances freezing tolerance. *Science* **280**, 104–106.

**Jasinski M, Sudre D, Schansker G, Schellenberg M, Constant S, Martinoia E, Bovet L.** 2008. AtOSA1, a member of the Abc1-like family, as a new factor in cadmium and oxidative stress response. *Plant Physiology* **147**, 719–731.

**Jonak C, Kiegl S, Ligterink W, Barker PJ, Huskisson NS, Hirt H.** 1996. Stress signaling in plants: a mitogen-activated protein kinase pathway is activated by cold and drought. *Proceedings of the National Academy of Sciences, USA* **93**, 11274–11279.

**Kang JY, Choi HI, Im MY, Kim SY.** 2002. Arabidopsis basic leucine zipper proteins that mediate stress-responsive abscisic acid signaling. *The Plant Cell* **14**, 343–357.

**Kasuga M, Liu Q, Miura S, Yamaguchi-Shinozaki K, Shinozaki K.** 1999. Improving plant drought, salt, and freezing tolerance by gene transfer of a single stress-inducible transcription factor. *Nature Biotechnology* **17**, 287–291.

**Kishor P, Hong Z, Miao GH, Hu C, Verma D.** 1995. Overexpression of [delta]-pyrroline-5-carboxylate synthetase increases proline production and confers osmotolerance in transgenic plants. *Plant Physiology* **108**, 1387–1394.

**Knight H, Knight MR.** 2001. Abiotic stress signalling pathways: specificity and cross-talk. *Trends in Plant Science* **6**, 262–267.

- Ko JH, Yang SH, Han KH.** 2006. Upregulation of an Arabidopsis RING-H2 gene, XERICO, confers drought tolerance through increased abscisic acid biosynthesis. *The Plant Journal* **47**, 343–355.
- Kobayashi Y, Yamamoto S, Minami H, Kagaya Y, Hattori T.** 2004. Differential activation of the rice sucrose nonfermenting1-related protein kinase2 family by hyperosmotic stress and abscisic acid. *The Plant Cell* **16**, 1163–1177.
- Krause G, Weis E.** 1991. Chlorophyll fluorescence and photosynthesis: the basics. *Annual Review of Plant Biology* **42**, 313–349.
- Leonard C, Aravind L, Koonin E.** 1998. Novel families of putative protein kinases in bacteria and archaea: evolution of the 'eukaryotic' protein kinase superfamily. *Genome Research* **8**, 1038–1047.
- Levitt J.** 1980. *Responses of plants to environmental stress: water, radiation, salt and other stresses*. New York: Academic Press.
- Li J, Wang XQ, Watson MB, Assmann SM.** 2000. Regulation of abscisic acid-induced stomatal closure and anion channels by guard cell AAPK kinase. *Science* **287**, 300–303.
- Lim PO, Kim Y, Breeze E, et al.** 2007. Overexpression of a chromatin architecture-controlling AT-hook protein extends leaf longevity and increases the post-harvest storage life of plants. *The Plant Journal* **52**, 1140–1153.
- Liu Q, Kasuga M, Sakuma Y, Abe H, Miura S, Yamaguchi-Shinozaki K, Shinozaki K.** 1998. Two transcription factors, DREB1 and DREB2, with an EREBP/AP2 DNA binding domain separate two cellular signal transduction pathways in drought- and low-temperature-responsive gene expression, respectively, in Arabidopsis. *The Plant Cell* **10**, 1391–1406.
- Ludwig A, Romeis T, Jones J.** 2004. CDPK-mediated signalling pathways: specificity and cross-talk. *Journal of Experimental Botany* **55**, 181–188.
- Marchler-Bauer A, Anderson JB, Chitsaz F, et al.** 2009. CDD: specific functional annotation with the conserved domain database. *Nucleic Acids Research* **37**, D205–D210.
- Mittler R, Vanderauwera S, Gollery M, Van Breusegem F.** 2004. Reactive oxygen gene network of plants. *Trends in Plant Science* **9**, 490–498.
- Mizoguchi T, Irie K, Hirayama T, Hayashida N, Yamaguchi-Shinozaki K, Matsumoto K, Shinozaki K.** 1996. A gene encoding a mitogen-activated protein kinase kinase kinase is induced simultaneously with genes for a mitogen-activated protein kinase and an S6 ribosomal protein kinase by touch, cold, and water stress in *Arabidopsis thaliana*. *Proceedings of the National Academy of Sciences, USA* **93**, 765–769.
- Mollet J, Delahodde A, Serre V, Chretien D, Schlemmer D, Lombes A, Boddaert N, Desguerre I, De Lonlay P, Ogier de Baulny H.** 2008. CAB31 gene mutations cause ubiquinone deficiency with cerebellar ataxia and seizures. *American Journal of Human Genetics* **82**, 623–630.
- Munnik T, Ligterink W, Meskiene I, Calderini O, Beyerly J, Musgrave A, Hirt H.** 1999. Distinct osmo-sensing protein kinase pathways are involved in signalling moderate and severe hyper-osmotic stress. *The Plant Journal* **20**, 381–388.
- Munns R.** 2002. Comparative physiology of salt and water stress. *Plant, Cell and Environment* **25**, 239–250.
- Mustilli AC, Merlot S, Vavasseur A, Fenzi F, Giraudat J.** 2002. Arabidopsis OST1 protein kinase mediates the regulation of stomatal aperture by abscisic acid and acts upstream of reactive oxygen species production. *The Plant Cell* **14**, 3089–3099.
- Olsen JV, Blagoev B, Gnadt F, Macek B, Kumar C, Mortensen P, Mann M.** 2006. Global, *in vivo*, and site-specific phosphorylation dynamics in signaling networks. *Cell* **127**, 635–648.
- Osakabe Y, Mizuno S, Tanaka H, Maruyama K, Osakabe K, Todaka D, Fujita Y, Kobayashi M, Shinozaki K, Yamaguchi-Shinozaki K.** 2010. Overproduction of the membrane-bound receptor-like protein kinase 1, RPK1, enhances abiotic stress tolerance in Arabidopsis. *Journal of Biological Chemistry* **285**, 9190–9201.
- Pandey S, Tiwari SB, Tyagi W, Reddy MK, Upadhyaya KC, Sopory SK.** 2002. A Ca<sup>2+</sup>/CaM-dependent kinase from pea is stress regulated and *in vitro* phosphorylates a protein that binds to AtCaM5 promoter. *European Journal of Biochemistry* **269**, 3193–3204.
- Poon W, Davis D, Ha H, Jonassen T, Rather P, Clarke C.** 2000. Identification of *Escherichia coli* ubiB, a gene required for the first monooxygenase step in ubiquinone biosynthesis. *Journal of Bacteriology* **182**, 5139–5146.
- Raichaudhuri A, Bhattacharyya R, Chaudhuri S, Chakrabarti P, Dasgupta M.** 2006. Domain analysis of a groundnut calcium-dependent protein kinase: nuclear localization sequence in the junction domain is coupled with nonconsensus calcium binding domains. *Journal of Biological Chemistry* **281**, 10399–10409.
- Riera M, Figueras M, Lopez C, Goday A, Pages M.** 2004. Protein kinase CK2 modulates developmental functions of the abscisic acid responsive protein Rab17 from maize. *Proceedings of the National Academy of Sciences, USA* **101**, 9879–9884.
- Saiki R, Ogiyama Y, Kainou T, Nishi T, Matsuda H, Kawamukai M.** 2003. Pleiotropic phenotypes of fission yeast defective in ubiquinone-10 production. A study from the abc1Sp (coq8Sp) mutant. *Biofactors* **18**, 229–235.
- Sakuma Y, Maruyama K, Osakabe Y, Qin F, Seki M, Shinozaki K, Yamaguchi-Shinozaki K.** 2006. Functional analysis of an Arabidopsis transcription factor, DREB2A, involved in drought-responsive gene expression. *The Plant Cell* **18**, 1292–1309.
- Salinas P, Fuentes D, Vidal E, Jordana X, Echeverria M, Holuigue L.** 2006. An extensive survey of CK2 alpha and beta subunits in Arabidopsis: multiple isoforms exhibit differential subcellular localization. *Plant and Cell Physiology* **47**, 1295–1308.
- Schippers JH, Nunes-Nesi A, Apetrei R, Hille J, Fernie AR, Dijkwel PP.** 2008. The Arabidopsis onset of leaf death5 mutation of quinolinate synthase affects nicotinamide adenine dinucleotide biosynthesis and causes early ageing. *The Plant Cell* **20**, 2909–2925.
- Shinozaki K, Yamaguchi-Shinozaki K.** 1997. Gene expression and signal transduction in water-stress response. *Plant Physiology* **115**, 327–334.
- Shinozaki K, Yamaguchi-Shinozaki K, Seki M.** 2003. Regulatory network of gene expression in the drought and cold stress responses. *Current Opinion in Plant Biology* **6**, 410–417.

- Singh K, Foley RC, Onate-Sanchez L.** 2002. Transcription factors in plant defense and stress responses. *Current Opinion in Plant Biology* **5**, 430–436.
- Taji T, Ohsumi C, Iuchi S, Seki M, Kasuga M, Kobayashi M, Yamaguchi-Shinozaki K, Shinozaki K.** 2002. Important roles of drought- and cold-inducible genes for galactinol synthase in stress tolerance in *Arabidopsis thaliana*. *The Plant Journal* **29**, 417–426.
- Takahashi Y, Nasir KH, Ito A, Kanzaki H, Matsumura H, Saitoh H, Fujisawa S, Kamoun S, Terauchi R.** 2007. A high-throughput screen of cell-death-inducing factors in *Nicotiana benthamiana* identifies a novel MAPKK that mediates INF1-induced cell death signaling and non-host resistance to *Pseudomonas cichorii*. *The Plant Journal* **49**, 1030–1040.
- Van Camp W, Capiou K, Van Montagu M, Inze D, Slioten L.** 1996. Enhancement of oxidative stress tolerance in transgenic tobacco plants overproducing Fe-superoxide dismutase in chloroplasts. *Plant Physiology* **112**, 1703–1714.
- Vert G, Chory J.** 2006. Downstream nuclear events in brassinosteroid signalling. *Nature* **441**, 96–100.
- Wang CX, Jing RL, Mao XG, Pang XB, Liu HM, Chang XP.** 2007. Cloning and expression analysis of a new stress-responsive gene *TaABC1L* in wheat. *Acta Agronomica Sinica* **33**, 878–884.
- Xiong L, Schumaker KS, Zhu JK.** 2002. Cell signaling during cold, drought, and salt stress. *The Plant Cell* **14**, Suppl, S165–S183.
- Yamaguchi-Shinozaki K, Shinozaki K.** 1994. A novel cis-acting element in an *Arabidopsis* gene is involved in responsiveness to drought, low-temperature, or high-salt stress. *The Plant Cell* **6**, 251–264.
- Yamaguchi-Shinozaki K, Shinozaki K.** 2006. Transcriptional regulatory networks in cellular responses and tolerance to dehydration and cold stresses. *Annual Review of Plant Biology* **57**, 781–803.
- Yoo SD, Cho YH, Tena G, Xiong Y, Sheen J.** 2008. Dual control of nuclear EIN3 by bifurcate MAPK cascades in C2H4 signalling. *Nature* **451**, 789–795.
- Zhang X, Wollenweber B, Jiang D, Liu F, Zhao J.** 2008. Water deficits and heat shock effects on photosynthesis of a transgenic *Arabidopsis thaliana* constitutively expressing ABP9, a bZIP transcription factor. *Journal of Experimental Botany* **59**, 839–848.
- Zhang Y, Yang C, Li Y, Zheng N, Chen H, Zhao Q, Gao T, Guo H, Xie Q.** 2007. SDIR1 is a RING finger E3 ligase that positively regulates stress-responsive abscisic acid signaling in *Arabidopsis*. *The Plant Cell* **19**, 1912–1929.