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OSABC1K8, AN ABC1-LIKE KINASE GENE, MEDIATES ABSCISIC ACID SENSITIVITY AND DEHYDRATION TOLERANCE RESPONSE IN RICE SEEDLINGS

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Abstract

The activity of bc1 complex kinase (ABC1K) protein family, which widely exists in prokaryotes and eukaryotes, consists of 15 members in rice, and the role of this family in plants has not yet been studied in details. In this study, a novel function of *OsABC1K8* (LOC_Os06g48770), a member of rice ABC1K family, was characterized. The transcript level of *OsABC1K8* changes in response to salt, dehydration, cold, PEG, oxidative (H_2O_2) stresses, or abscisic acid (ABA) treatment. Overexpression of *OsABC1K8* significantly increased sensitivity to dehydration and reduced sensitivity to ABA. In the contrast, RNAi transgenic lines displayed significantly reduced sensitivity to dehydration stress and increased sensitivity to ABA. Furthermore, the transcriptional levels of several ABA/stress-regulated responsive genes were suppressed in *OsABC1K8* over-expressing plants under dehydration stress. In conclusion, our results suggested that *OsABC1K8* is a negative regulator in response to dehydration stress through an ABA-dependent pathway.

Keywords: Rice, ABC1Ks, ABA sensitivity, Dehydration stress.

Abbreviations: ABA = Abscisic acid, GFP = Green fluorescent protein, *Hyg* = *hygromycin,* ORF = Open reading frame, PEG = Polyethyleneglycol, qRT-PCR = Real-time quantitative RT-PCR, RWC = Relative water content, TF = Transcription factor

Introduction

Plants are always exposed to various abiotic stresses caused by environmental changes during their life cycle. Water deficit is one of the most important environmental factors that affect plants growth, development, and crops production (Cattivelli *et al.*, 2008). For the reason that rice is a semiaquatic species typically cultivated under partially flooded conditions, it is more susceptible to water deficit stress. Therefore, the improvement of water deficit tolerance may increase actual yields in rice production.

Plants modulate adaptive responses to water deficit through a series of complex signaling pathways. Drought-responsive factors, including transcription factors (TFs), protein kinases and other drought related genes, are induced in an abscisic acid (ABA)-dependent or -independent manner in rice under drought conditions (Deikman *et al.*, 2012; Hadiarto & Tran, 2011). Several TFs such as members of the Dehydration Responsive Element Binding Protein (DREB), bZIP, MYB and NAC TF families have been well characterized with their roles in transcriptional regulation of acclimation responses and tolerance to drought (Fukao & Xiong, 2013; Todaka *et al.*, 2012). Recent studies on rice protein kinases, including members of MAPK, CDPK and SnRK subfamilies, revealed the phosphorylation pathways related to drought stress (Fujita *et al.*, 2009; Ning *et al.*, 2010; Rohila & Yang, 2007). Furthermore, dozens of rice genes such as *LEAs* and *RAB16* have been identified as drought-responsive genes (Hadiarto & Tran, 2011).

The ABC1K (activity of bc1 complex kinase), a new group of atypical protein kinases, is an evolutionarily ancient gene family, conserved throughout species of archaea, bacteria and eukaryotes (Bousquet *et al.*, 1991; Leonard *et al.*, 1998; Lundquist *et al.*, 2012). The first *ABC1K* gene was identified as an essential factor for cytochrome b mRNA translation and electron transfer in the *bc1* complex in *Saccharomyces cerevisiae* (Bousquet *et al.*, 1991). Further studies in yeast, *Escherichia coli* and human suggested that ABC1K proteins are not only essential for ubiquinone biosynthesis but also for the proper conformation and efficient functioning of the bc1 complex and neighboring complexes in the respiratory chain (Brasseur *et al.*, 1997; Do *et al.*, 2001; Hsieh *et al.*, 2004; Leonard *et al.*, 1998; Xie *et al.*, 2011). The conserved role of this protein family in plants was uncovered from a complementation result that the activity of complex III was partially restored by expression of *AtABC1K13* in the ABC1 mutant yeast (Cardazzo *et al.*, 1998). However, proliferation of the ABC1K family in plants suggests an expansion of targets and functions (Lundquist *et al.*, 2012). Previous work showed that most of the ABC1 proteins in plants are involved in abiotic stress responses. For instance, AtACDO1 (ABC1K1) is critical in chlorophyll degradation and response to photooxidative stress (Yang *et al.*, 2012b). ZmABC1-10 is a cadmium-responsive factor and may play potential roles in plants adaption to ABA, H_2O_2, and darkness stresses (Gao *et al.*, 2010). TaABC1 confers enhanced tolerance to abiotic stresses in *Arabidopsis* and is involved in hypersensitive response against the stripe rust fungal pathogen (Wang *et al.*, 2011). A chloroplast ABC1K protein AtOSA1 was identified as a factor playing a role in the balance of oxidative stress (Jasinski *et al.*, 2008). AtSIA1 (salt-induced ABC1 kinase 1) is a chloroplast-localized protein tightly associated with the thylakoid membranes and contributes to oxidative stress response and isoprenyl lipid synthesis (Yang *et al.*, 2012). Recently, the function of two plastoglobule-localized ABC1K members

ABC1K1 and ABC1K3 are involved in plastoglobule prenyl-lipid metabolism and chloroplast morphology in *Arabidopsis* (Lundquist *et al.*, 2013; Martinis *et al.*, 2013).

Recent results showed that there are 15 putative ABC1 genes in rice (Gao *et al.*, 2011; Lundquist *et al.*, 2012; Yang *et al.*, 2012a). The transcription analysis of rice ABC1K genes suggested their expression is highest in leaf tissue and responses to abiotic stresses (Gao *et al.*, 2011; Yang *et al.*, 2012a). However, most ABC1K genes in rice have not yet been investigated in details and their roles remain unclear. In this study, we characterized the novel function of a rice ABC1K gene *ABC1K8* under dehydration stress and ABA signaling response. The expression of *ABC1K8* is varied in different stress treatments. The transgenic plants with over-expression of *ABC1K8* exhibit reduced dehydration tolerance and enhanced sensitivity to ABA, whereas the *ABC1K8* knock-down plants displayed opposite phenotypes. The expression levels of ABA and stress related genes are different between the transgenic and wild type plants under dehydration condition. These results provided evidence that *OsABC1K8* plays a significant role in regulation of dehydration stress response in rice seedlings.

Materials and Methods

Plant materials, growth conditions and treatment: Both the transgenic and wild type (WT) rice plants used in this study were *Oryza sativa* L. ssp. *japonica* cv. Zhonghua 11, ZH11. Seeds were dehulled and surface-sterilized with 75% ethanol for 90 seconds, then with 2.5% sodium hypochlorite for 45 min and washed extensively with distilled water for 5 times. Sterilized seeds were grown on MS medium in a growth chamber at 28-30^{o}C in a light/dark cycle of 16 h/8 h for two weeks. Then, the rice plants were grown in a greenhouse at 30^{o}C during the day and 20^{o}C at night in a light/ dark cycle of 14 h/10 h.

To measure the transcript level of the *OsABC1K8* under abiotic stress and hormone treatment, rice seeds were germinated and grown in Kimura B solution at 28°C for 10 days in a growth chamber with 16/8 h light/dark cycle. Then, the seedlings were transplanted in solutions containing 300 mM NaCl, 1% H_2O_2 (100 μM), 20% PEG 6000 and 100 μM ABA, respectively. For ABA and PEG treatments, the seedlings were sampled at 0, 4, 6, 12, and 24 h after treatment. For H_2O_2 and NaCl treatments, the seedlings were sampled at 0, 1, 4, 6, and 12 h after treatment. For cold stress, seedlings were transferred to a growth chamber at 4°C and sampled at 0, 4, 6, 12, and 24 h after treatment. Dehydration stress was realized by putting intact plants in the air without water supply, and plant leaves were sampled at 0, 1, 3, 5, and 7 h after treatment. These collected samples were immediately frozen in liquid nitrogen and stored at - 80^{o}C for RNA extraction.

Plasmid construction and transgenic plant generation: To generate the *OsABC1K8* overexpression constructs, the full-length cDNA of *OsABC1K8* was amplified by RT-PCR from total RNA of Zhonghua11 using primers P1 and P2 (Table S1). The PCR products were inserted into the plant binary vector pCAMBIA1390 under the control of the maize Ubi promoter. The RNAi construct was constructed as previously described by Luo *et al.* (2005). A cDNA fragment of *OsABC1K8* (663bp to 1118 bp) from ZH11 was cloned using primers P3 and P4 (Table S1) and inserted into the binary vector pYLRNAi. Both constructs were introduced into *Agrobacterium tumefaciens* strain EHA105 and transferred into Zhonghua11. Seedlings were examined by PCR at the DNA and RNA levels in the T1 generation; T2 and T3 (homozygous lines) seeds were screened by germinating with the selection of 50 mg/L hygromycin. Homozygous T3 generation of transgenic rice plants were used in subsequent experiments.

Table S1. All primer sequences used in this study.

Primers	Sequences (5'-3')
P1	5'-GAATTCCTACTCCGGCGGCGCGAGGTTG-3'
P2	5'-ACTAGTTGTGCTGACTTCTAATGGGAT-3'
P3	5'-AGATCTATGGGAAACACCTTAACCAG-3'
P4	5'-ACTAGTCTCCGGCGGCGCGAGGTTGAG-3'
OsLEA3	F: 5'-GCCGTGAATGATTTCCCTTTG-3' R: 5'-CACACCCGTCAGAAATCCTCC-3'
OsRAB16A	F: 5'-CATGGACAAGATCAAGGAGAAGC-3' R: 5'-CTTATTATTCAGGAAGGTGACGTGG-3'
OsLIP9	F: 5'-TGGAATTTGGAAGTGTTTGGC-3' R: 5'-CCCACACGAAACACAAACTTC-3'
OsP5CS1	F: 5'-TGTCTTGGCTTCAAATAGCGG-3' R: 5'-GCGGCAACAGCCATCTCAC-3'
OsABI2	F: 5'-AATCGTTGTGAGGAAGTG-3' R: 5'-CGTGAGGCTTATAGTTGTTA-3'
OsbZIP12	F: 5'-GAGAACGCCAAGATGTTCAA-3' R: 5'-TCTCGTGCTGACGTTTTCC-3'
OsbZIP23	F: 5'-GGGCATCGAGAAGGTTGT-3' R: 5'-GAGTCCTCCGAAGGCAAAT-3'
OsbZIP46	F: 5'-ATCAAGAACAGGGAGTCCGC-3' R: 5'-GAGCCATCACCATTCACCAA-3'
OsbZIP72	F: 5'-AATGAGGTAGAAGAAATGAT-3' R: 5'-GCACAGTCGCTGATGAAGG-3'

RNA extraction and quantitative real-time PCR: Total RNA of various tissues from Zhonghua 11 transgenic rice lines and WT control were extracted with Trizol reagent (Invitrogen, Inc.) according to the manufacturer's instructions. cDNAs were synthesized from 1μg of total RNA using a Prime Script RT reagent Kit (Perfect Real Time) (Takara Bio, Inc.). The qRT-PCR was analyzed by means of real-time PCR and it was performed using the Bio-Rad with SYBR Premix Ex Taq (TaKaRa Bio, Inc.). The PCR program was: 95^{o}C, 30 s; 95^{o}C, 5 s; 56^{o}C, 34 s; 40 cycles. The melting curve was acquired at the end. Rice *OsActin1* gene was used as an internal control. The relative expression level was calculated by $2^{\Delta\Delta\text{-Ct}}$ (Livak & Schmittgen, 2001). Each experiment was performed with three replicates. All of the above experiments were carried out using the corresponding manufacturer's instructions, and all the gene-specific primers are listed in Table S1.

Dehydration stress tolerance and ABA response analysis of WT and transgenic lines: The dehydration stress tolerance was analyzed as previously described by Lu *et al.* (2009), rice seeds were germinated on MS agar medium for 7 days, and then grown in Kimura B solution culture for 7 days in a growth chamber. Two-week-old rice seedlings were placed on a bench in the growth chamber, whole plants were exposed to air for 5 h, followed by 4-day recovery incubated in Kimura B solution. The survival rates were then recorded. The data were from three independent experiments (16 seedlings from each line were used in each experiment).

The water loss rate and relative water contents (RWC) were measured essentially by following the already reported methods (Xiang *et al.*, 2008; Zhang *et al.*, 2012). Briefly, leaves of two-week old homozygous transgenic and WT seedlings were cut and weighed fresh weight immediately (FW). Then, the detached leaves were placed on a bench at room temperature to induce dehydration and weighed at 0.5, 1, 3, 5, 7 and 9 h (desiccated weight).

$$\text{Water loss rate (\%)} = (\text{FW} - \text{desiccated weight})/\text{FW} \times 100.$$

The dehydrated leaves were soaked in distilled water for 4 h and turgid weight (TW) was recorded. Leaves were finally dried for 48 h at 80°C to obtain total dry weight (DW). RWC was calculated as follows:

$$\text{RWC (\%)} = [(\text{desiccated weight} - \text{DW})/(\text{TW} - \text{DW})] \times 100.$$

All testing experiments for stress tolerance were performed in triplication.

H_2O_2 was detected by DAB staining according to Ouyang *et al.* (2010). Plant leaves were excised and immersed in 1% solution of DAB in Tris-HCl buffer (pH 3.8). After vacuum infiltration for 20 min, the samples were incubated at room temperature for 20 h in the dark. When the brown spots appeared clearly, leaves were bleached by immersing in boiling ethanol for 20 minutes to visualize the brown spots. The brown spots were characteristic of the reaction of DAB with H_2O_2.

For ABA treatments, seedlings were germinated on MS medium at 26°C for 2 days. Then uniformly germinated seeds were transferred to MS medium with 0μM and 4μM ABA for 9 days, respectively, normal MS medium as controls After treatment, rice seedlings were collected to examine root and shoot length and fresh weight. The data were from three replicates (10 seedlings in each replicate).A Student's t-test was used for statistical analysis.

Bioinformatics analysis: The protein sequences of ABC1Ks were used to search against the japonica rice annotation database (http://rice.plantbiology.msu.edu/). The phylogenetic tree was constructed with Neighbor-joining method with bootstrapping analysis by MEGA5.0 (Tamura *et al.*, 2011). Other gene sequences were blasted from The National Center for Biotechnology Information (NCBI).

Result

Phylogenetic analysis and expression patterns of *OsABC1K8*: The expression profile of rice ABC1K gene family including 15 members was previously analyzed under drought stress treatment by qRT-PCR (Gao *et al.*, 2011). Among the down-regulated genes, *OsABC1K8* (LOC_Os06g48770) was further characterized.

OsABC1K8 encoded a putative protein of 948 amino acids with a calculated molecular mass of 104.3 kDa. To investigate the evolutionary relationship of OsABC1K8 among the ABC1K protein family, a phylogenetic tree was constructed based on the amino acid sequences of the rice ABC1Ks, and some previously reported ABC1K proteins from other plants and yeast (Fig. 1A). According to the analysis, OsABC1K8 may be divided into a separate subgroup and had a relatively closer relationship with AtABC1.

The expression pattern of *OsABC1K8* in different tissues was analyzed by qRT-PCR using the rice Zhonghua 11 grown under a normal condition (Fig. 1B). The result showed that *OsABC1K8* is expressed in all tested tissues, especially with a higher level in leaves.

To detect the effects of abiotic stress and phytohormone on the expression of *OsABC1K8*, various treatments were applied to rice seedlings (*O. sativa*, Zhonghua 11) including NaCl (300 mM), dehydration, cold (4°C), H_2O_2 (100 μM), 20% PEG and ABA (100 μM) (Fig. 1C), and the RNA was extracted for qRT-PCR. The expression of *OsABC1K8* was gradually repressed by dehydration and PEG with the extension of treatment time. In ABA treatment, *OsABC1K8* expression was shown decreased at the time of initiation with a following induction and suppression in longer treatment. For H_2O_2 stress, the *OsABC1K8* expression level was quickly decreased after treatment, and then slightly increased. The expression level of *OsABC1K8* was induced slightly at the initiation of cold treatment and suppressed to the lowest level at 24 h after treatment. *OsABC1K8* transcription was also induced to the highest level at 12 h after NaCl treatment. Collectively, the expression of *OsABC1K8* is regulated in responses to multiple abiotic stresses and ABA.

Generation of *OsABC1K8* transgenic rice lines: To further characterize the function of *OsABC1K8*, we generated transgenic plants over-expressing *OsABC1K8* under a ubiquitin (Ubi) promoter and *OsABC1K8* RNAi transgenic lines by over-expressing inverted repeat fragments separated by an intron (Fig. 2A). By qRT-PCR analysis, two independent homozygous lines OE4-10 and OE7-2 showed around 7.0 and 7.5 fold increases, respectively, compared to that of wild type (Fig. 2B). Relative expression levels of two *OsABC1K8* RNAi plant lines were 17% and 18% compared to that of WT plants (Fig. 2B). There was no obvious difference in morphology between the transgenic plants and WT (ZH11) under normal conditions (Fig. 2C).

***OsABC1K8* transgenic rice seedlings display different sensitivity to dehydration stress:** In order to evaluate the dehydration stress tolerance of *OsABC1K8*, the whole seedlings of 14-day-old transgenic and WT plants were exposed to air for 5 h followed by rehydration for 4 days. Before dehydration treatment, the transgenic lines showed no significant difference in growth compared with WT controls (Fig. 3A, upper left panel). After treatment, the leaves of overexpressing lines exhibited early withering phenotype, compared with other plants (Fig. 3A, upper right panel). After rewatering for 4 days, the *OsABC1K8*-RNAi lines were taller and stronger then WT plants. By contrast, the over-expressing lines were not recovered from dehydration stress (Fig. 3A).

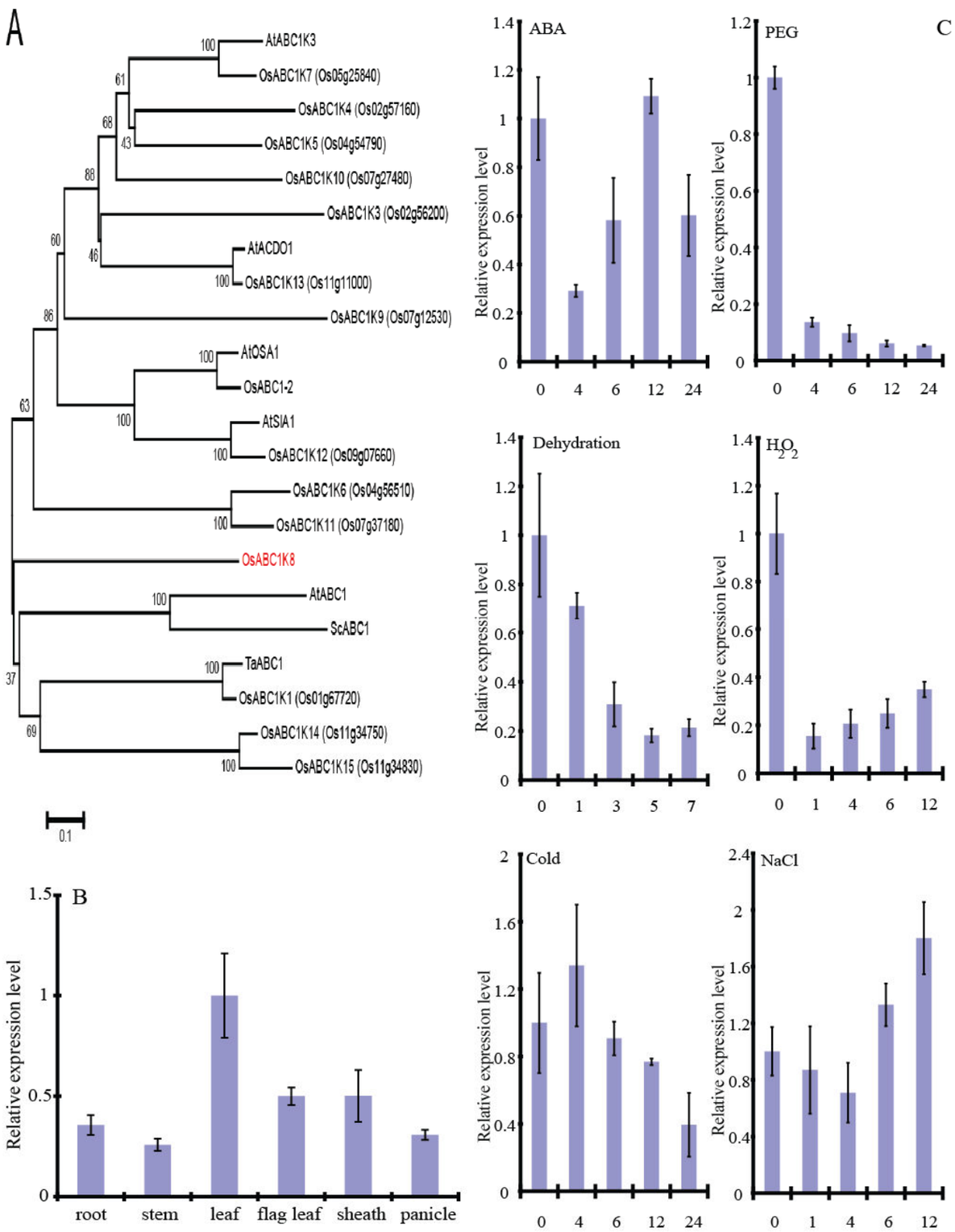

Fig. 1. Phylogenetic tree analysis of OsABC1K8 and expression analysis of the *OsABC1K8* gene.
(A) Phylogenetic tree of ABC1K proteins. The phylogenetic tree was constructed in MEGA5.0 software with the neighbor-joining method. The numbers indicate the bootstrap values (1,000 replications). Scale indicates amino acid substitutions per position. At, *Arabidopsis*; Os, *Oryza sativa* L.; Ta, *Triticum aestivum* L. Sc, *Saccharomyces cerevisiae*. **(B)** qRT-PCR analysis of the expression level of *OsABC1K8* in different tissues of Zhonghua11. **(C)** Expression patterns of *OsABC1K8* under various stress treatments including ABA (100 μM), 20% PEG, dehydration, H_2O_2 (100 μM), cold (4°C) and NaCl (300 mM). Relative expression levels of *OsABC1K8* were examined by qRT-PCR. Error bars indicate Standard Deviation (SD) based on 3 replicates.

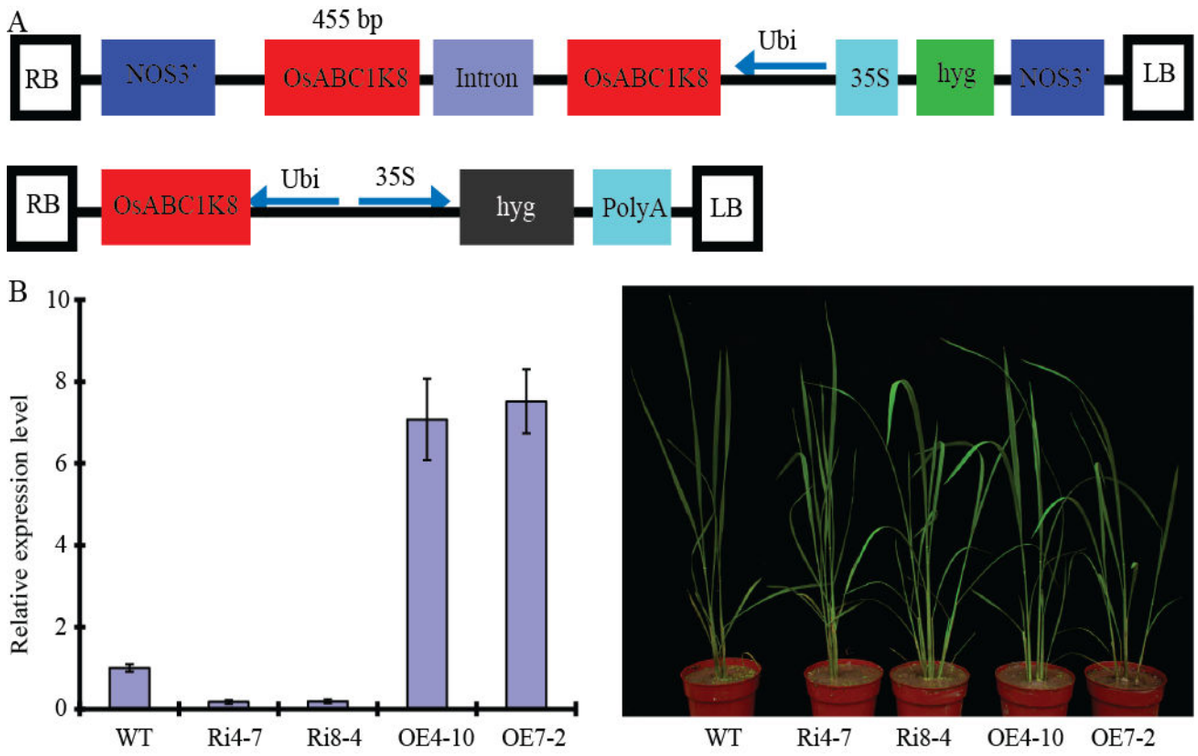

Fig. 2. Generation of the *OsABC1K8* overexpressing and RNAi transgenic plants.
(A) Diagram of the plant expression vector pYLRNAi-OsABC1K8 used for *OsABC1K8* knockdown, and pHQSN-*OsABC1K8* used for *OsABC1K8* overexpression in transgenic plants. **(B)** *OsABC1K8* expression in T3 transgenic lines as determined by qRT-PCR. "Ri" indicates RNAi transgenic lines and "OE" indicates overexpressing transgenic lines. **(C)** Phenotypes of 6-week-old WT, RNAi and overexpressing lines.

The survival rates of *OsABC1K8*-RNAi lines Ri4-7 and Ri8-4 (92% and 91%, respectively) were higher than that of WT (77%). However, *OsABC1K8*-overexpression lines OE4-10 and OE7-2 showed very low survival rates (15% and 27%, respectively) (Fig. 3B). In addition, the leaves from *OsABC1K8*-overexpressing seedling had higher water loss rate and RWC, compared with leaves from WT during dehydration, and the leaves of RNAi transgenic plants showed an opposite performance (Fig. 3B). Dehydration promotes rapid accumulation of ROS, such as H_2O_2, which function as signal to trigger diverse acclimation responses to stress (Blokhina & Fagerstedt, 2010; Fukao *et al.*, 2011). So we examined whether *OsABC1K8* plays a role in stress tolerance through detoxification of ROS. The leaves of transgenic and WT lines were stained by 3,3 ¢-diamino benzidine (DAB) to detect H_2O_2 levels. As shown in Fig. 3C, under normal conditions, the DAB staining spot was not observed in the leaves of all plant lines. After dehydration treatment for 5 h, very few brown staining spots were shown within the total leaves of RNAi plants, whereas some areas of the leaves in control plants became brown. Interestingly, the leaves of *OsABC1K8* overexpressing lines exhibited much stronger DAB staining than those of the corresponding control plants. Taken together, these findings suggested that the overexpression of *OsABC1K8* in rice resulted in enhanced sensitivity to dehydration while RNAi lines of the *OsABC1K8* reduced dehydration sensitivity at the seedling stage.

***OsABC1K8* transgenic rice plants show different sensitivity to exogenous ABA:** Generally, increased tolerance to dehydration is accompanied with hypersensitivity to ABA in plants (Fukao *et al.*, 2011). Since *OsABC1K8* regulates dehydration tolerance, an interesting question is whether the *OsABC1K8* transgenic plants display different sensitivity to ABA. Firstly, seeds were germinated on solid MS containing 0, 4 or 8 µM ABA. However, the germination rates of the *OsABC1K8* transgenic lines and wild type seeds showed no significant difference (data not shown). Next, we examined the seedlings for ABA sensitivity at the post-germination stage using media with and without 4 µM ABA. Under normal conditions, the growth phenotypes of transgenic and WT seedlings showed no significant difference (Fig. 4A). However, the root and shoot growth in RNAi plants was inhibited more severely by ABA, compared with that in the WT plants. By contrast, *OsABC1K8*-overexpressing seedlings were healthier and stronger than WT seedlings under 4 µM ABA treatment (Fig. 4B). Moreover, the fresh weight of *OsABC1K8*-overexpressing seedlings was higher than that of WT or *OsABC1K8* RNAi lines (Fig. 4B). This result indicated that *OsABC1K8*-overexpressing plants showed decreased ABA sensitivity while RNAi transgenic plants displayed increased ABA sensitivity at the post-germination stage, implying that *OsABC1K8* might negatively regulate rice response to ABA signaling.

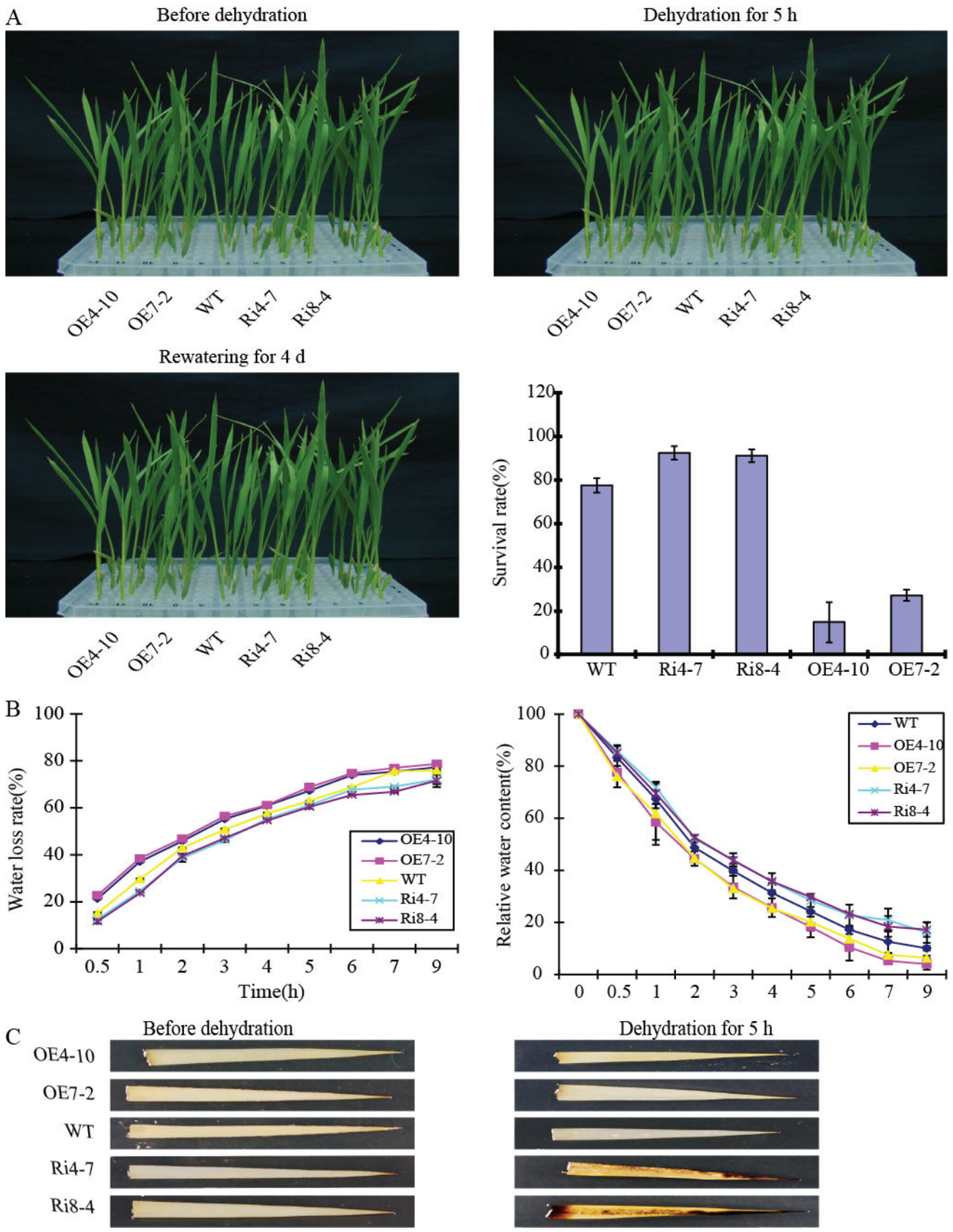

Fig. 3. Performance of *OsABC1K8* transgenic plants under dehydration stress.
(A) Photographs of transgenic lines and controls after dehydration treatment. Two-week-old rice seedlings were deprived of water for 5 hours, followed by recovery for 4 days. Photos of transgenic lines and WT were taken at these time points, and then the survival rates were calculated. **(B)** Water loss rate and relative water content of detached leaves from WT and transgenic lines at two-week-old during the dehydration process. Data are means ± SD of three independent experiments. **(C)** 3,3 ¢-Diaminobenzidine (DAB) staining in leaves of two-week-old rice seedlings under dehydration stress. DAB staining of rice leaves from plants before dehydration (CK, left panel) or dehydration treatment for 5 hours (right panel). The brown region on leaves indicates the H_2O_2 level.

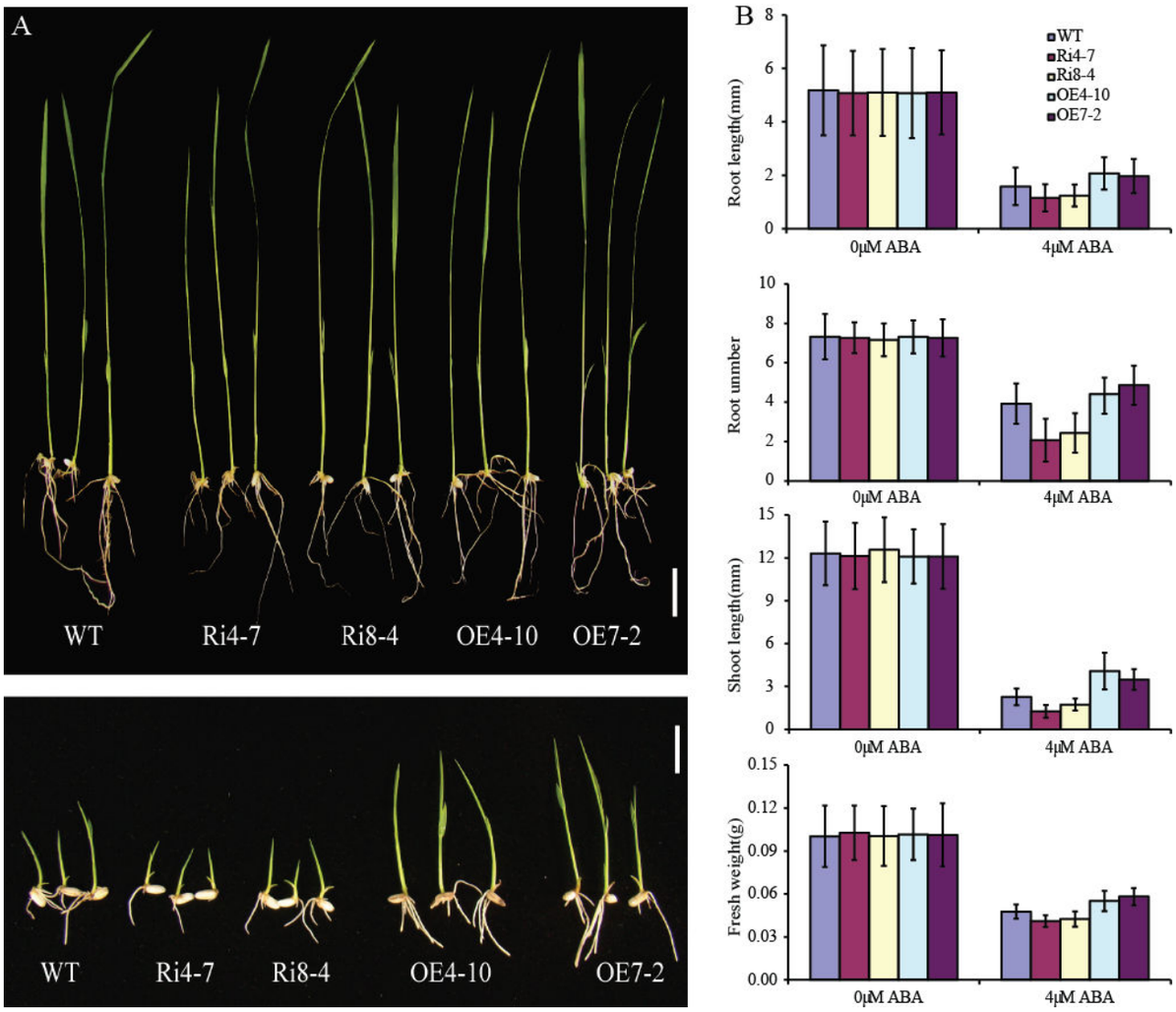

Fig. 4. ABA sensitivity assays of *OsABC1K8* Overexpression and RNAi rice lines.
(A) Phenotypic comparison of rice Seedlings were transferred to MS medium with 0μM and 4μM ABA for 9 days. (**B)** Shoot length, root length, root number and fresh weight of rice seedlings harvested after treatment for 9 days. Data are means ± SD from three independent experiments. Asterisks indicate significant difference between the WT and transgenic lines (*p<0.05).

***OsABC1K8* regulates ABA- and stress-responsive genes under dehydration stress:** Acclimation responses to drought are properly coordinated through integrated regulatory networks that consist of ABA-dependent and -independent pathways (Fukao *et al.*, 2011; Nakashima *et al.*, 2009). To further reveal the regulatory mechanism of *OsABC1K8* in dehydration stress response, transcript levels of several well-characterized ABA and stress responsive genes were checked in the *OsABC1K8* transgenic and WT rice seedlings under dehydration stress condition. The examined genes included *OsLEA3* (LOC_Os06g21910), encoding a late embryogenesis abundant protein, one of osmotic adjustment-related genes(Moons *et al.*, 1997); *Rab16A* (LOC_Os11g26790), encoding a basic glycine-rich protein (Yamaguchi-Shinozaki *et al.*, 1990); *OsABI2* (LOC_Os01g40094), an ABA signaling gene belonging to PP2C family (Park *et al.*, 2010); *OsLIP9* (LOC_Os02g44870), a low temperature-induced gene (Aguan *et al.*, 1991); *OsP5CS1* (LOC_Os05g38150), encoding Δ1-pyrroline-5-carboxylate synthetase involved in proline biosynthesis (Hong *et al.*, 2000); *OsbZIP12*(LOC_Os01g647300), *23* (LOC_Os02g52780), *46* (LOC_Os06g10880), and *72* (LOC_Os09g28310) encoding bZIP (basic leucine zipper) transcription factors (Joo *et al.*, 2014; Tang *et al.*, 2012).

Under normal conditions, the transcript level of *OsABI2* was higher in *OsABC1K8*-overexpression lines and lower in *OsABC1K8*-RNAi lines, compared with that in WT. The transcript levels of *OsLEA3*, *OsABI2*, and *OsbZIP23* were lower in *OsABC1K8*-RNAi lines than in WT (Fig. 5). After dehydration treatment, the transcript levels of *OsLEA3*, *OsP5CS1*, *OsRab16A*, and *OsbZIP46* were higher in *OsABC1K8*-RNAi than in WT. However, the expression levels of all selected genes except *OsbZIP12* and *OsbZIP72* were extremely lower in *OsABC1K8*-overexpression plants than those in the control and *OsABC1K8*-RNAi lines (Fig. 5). These results indicated that *OsABC1K8* has effects on the transcription of ABA- and stress-associated genes in dehydration stress.

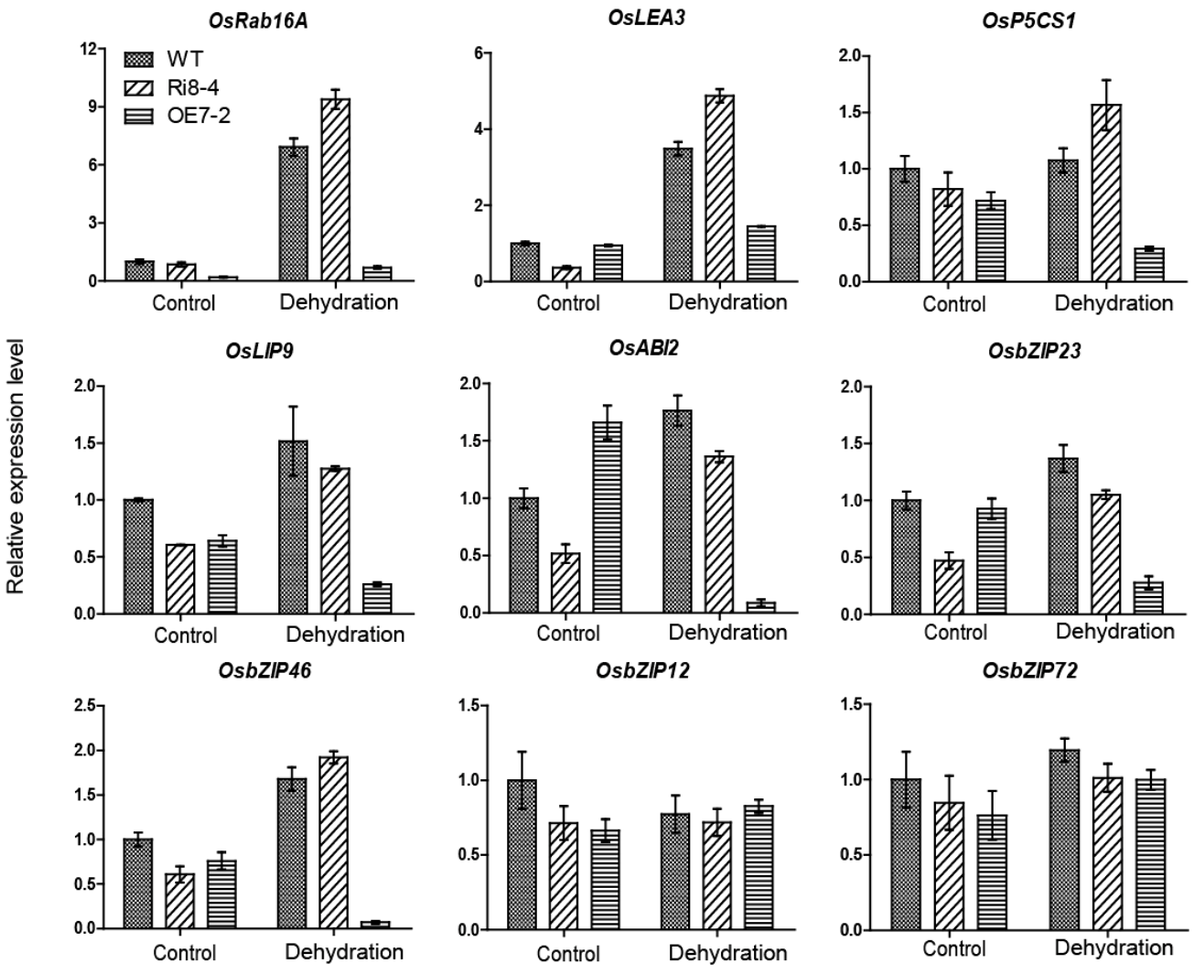

Fig. 5. Expression analysis of selected ABA- and stress-responsive genes in *OsABC1K8* transgenetic lines under dehydration treatments. Two-week-old rice seedlings were treated without (normal conditions) or with air dried for 4 h. Leaves were collected to detect transcript levels of those ABA- and stress-responsive genes. The mRNA fold difference is relative to that of WT samples under normal conditions. Data are means ± SD of three independent experiments.

Discussion

Previous studies showed that there are 15 putative ABC1K genes in rice by bioinformatics prediction and most of them are responsive to abiotic stresses in systematic expression analysis (Gao *et al.*, 2011; Yang *et al.*, 2012a). Our phylogenetic result indicated that OsABC1K8 belongs to the ABC1K family, and OsABC1K8 is not clustered into the same group with the ABC1 proteins which previously reported that located at mitochondria or chloroplast but was divided into a separate subgroup (Fig. 1A). These results suggested that *OsABC1K8* may play a novel function in rice. In recent years, many experimental studies of plant ABC1K genes have showed that this gene family is involved in various types of abiotic stress tolerance. For instance, *OsABC1-2* confers enhanced tolerance to dark-induced stress in rice (Gao *et al.*, 2012). Our present study demonstrated that the expression of *OsABC1K8* is suppressed by multiple abiotic stresses including dehydration, PEG and H_2O_2 stresses (Fig. 1B), implying that *OsABC1K8* might be a new stress responsive gene participating in rice responses to multiple stresses.

Dehydration in plants leads to growth disruption and results in cell death in severe cases, so plants have to evolve an adaption mechanism in this stress condition (Ali *et al*., 2007; Deikman *et al.*, 2012). Our results from *OsABC1K8* transgenic plants showed that knockdown lines exhibit increased dehydration tolerance while over-expresion lines display hypersensitivity to dehydration. The data from relative water loss rate suggested that the water maintenance in leaves is regulated by the expression level of *OsABC1K8*. Because the short term response to dehydration may be mediated by stomatal closure, and *OsABC1K8* expression level affect H_2O_2 content (Fig. 3B), it is possible that *OsABC1K8* is associated with H_2O_2-induced stomatal movement. Dehydration tolerance always accompanies hypersensitivity to ABA in plants, and our ABA treatment results are consistent with this general rule. The *OsABC1K8*-overexpression plants are insensitive to ABA, opposite to their actions response to dehydration. Accumulation of ABA increased under drought stress, results in enhanced hydraulic conductivity in vascular system and stomatal closure to improve water uptake and

retention (Mishra *et al.*, 2006; Wilkinson & Davies, 2002). Since the *OsABC1K8* RNAi plants show more sensitive to exogenous ABA (Fig. 4B), so the cells in the *OsABC1K8* RNAi plants may also be more sensitive to increased endogenous ABA and take a much faster action to maintain water content under dehydration condition. Meanwhile, because of their insensitivity to ABA, the response to dehydration in the over-expression plants would be corresponding slower which causes low survival rate in the dehydration treatment.

Our results from ABA signaling and stress response gene expression profile under dehydration treatment provided further evidence that *OsABC1K8* plays its function in dehydration response mediated by an ABA dependent pathway at seedling stage. Proline synthesis enzyme P5CS and Late embryogenesis abundant (LEA) proteins are always upregulated in drought and ABA treatments and play functions in cell protection during osmotic stress. Our result displayed that the expression levels of *P5CS* and three *LEA* genes, including *LEA3*, *RAB16A* and *LIP9*, are downregulated in response to dehydration. The rice ABF/AREB transcription factors homologs OsbZIP12, 23, 46 and 72 were well characterized as important players in the ABA signaling pathway under drought stress in rice (Lu *et al.*, 2009; Tang *et al.*, 2012; Xiang *et al.*, 2008). Interestingly, the expression levels of *OsbZIP23* and *46* are dramatically suppressed in *OsABC1K8* overexpression plants under dehydration treatment, suggesting that the expression of genes involved in an early step of ABA pathway may be also impaired in *OsABC1K8* transgenic plants (Fig. 5). The expression pattern of the maker genes in *OsABC1K8* transgenic plants was similar to *OsDREB6* under PEG treatment, which showed that the expression level of *OsDREB6* affects the ABA signal transduction pathway to respond to abiotic stress (Ke *et al.*, 2014). Taken together, the ABA signaling in dehydration response is suppressed by accumulation of *OsABC1K8*, confirming our conclusion that *OsABC1K8* regulates dehydration tolerance by ABA dependent pathways.

Because our expression analysis showed that the transcript profiles of ABA induced genes is different in *OsABC1K8* transgenic plants in dehydration condition, but OsABC1K8 is a protein kinase, the question is how this kinase acts on signal transduction during dehydration response. Upstream of transcription regulation, various signal transduction systems function in abiotic stress responses, involving protein phosphorylation and dephosphorylation, phospholipid metabolism, calcium sensing, protein degradation and so on (Bartels & Sunkar, 2005; Umezawa *et al.*, 2006). Numerous studies have showed that protein phosphorylation exerts a central role in mediating drought response (Christmann *et al.*, 2006; Xiong *et al.*, 2002; Zhang *et al.*, 2012). For example, the SAPK8, SAPK9 and SAPK10 kinases are able to directly phosphorylate the rice ABRE-binding TRAB1 in response to ABA, suggesting that the regulation of bZIP TFs by SnRK2 kinases is conserved among plant species (Kobayashi *et al.*, 2005; Nakashima *et al.*, 2009; Umezawa *et al.*, 2006). Similarly, the potential working model for *OsABC1K8* is regulation of signaling component in ABA and dehydration responsive pathway by phosphorylation. The substrates of OsABC1K8 need to be identified in the further experiment to uncover the molecular function of this kinase.

Conclusions

We characterized the role of *OsABC1K8* in dehydration tolerance and ABA sensitivity regulation, *OsABC1K8* may play a negative role in regulation of dehydration stress through an ABA-dependent manner. These results would help us to understand the molecular mechanism of abiotic stress response in rice.

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Reference

Aguan, K., K. Sugawara, N. Suzuki and T. Kusano. 1991. Isolation of genes for low-temperature-induced proteins in rice by a simple subtractive method. *Plant and Cell Physiol.,* 32(8): 1285-1289.

Ali, Q., M. Ashraf and H.U.R. Athar. 2007. Exogenously applied proline at different growth stages enhances growth of two maize cultivars grown under water deficit conditions. *Pak. J. Bot.,* 39(4): 1133-1144.

Bartels, D. and R. Sunkar. 2005. Drought and salt tolerance in plants. *Critic. Rev. Plant. Sci.,*24(1): 23-58.

Blokhina, O. and K.V. Fagerstedt. 2010. Oxidative metabolism, ROS and NO under oxygen deprivation. *Plant. Physiol. Biochem.,* 48(5): 359-373.

Bousquet, I., G. Dujardin and P.P. Slonimski. 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 .J.,* 10(8): 2023.

Brasseur, G., P. Tron, G. Dujardin, P.P. Slonimski and P. Brivet-Chevillotte. 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. *Eur. J. Biochem.,* 246(1): 103-111.

Cardazzo, B., P. Hamel, W. Sakamoto, H. Wintz and G. Dujardin. 1998. Isolation of an *Arabidopsis thaliana* cDNA by complementation of a yeast abc1 deletion mutant deficient in complex III respiratory activity. *Gene*, 221(1): 117-125.

Cattivelli, L., F. Rizza, F.W. Badeck, E. Mazzucotelli, A.M. Mastrangelo, E. Francia, C. Mare, A. Tondelli and A.M. Stanca. 2008. Drought tolerance improvement in crop plants: An integrated view from breeding to genomics. *Field. Crops. Res.,* 105(1): 1-14.

Christmann, A., D. Moes, A. Himmelbach, Y. Yang, Y. Tang and E. Grill. 2006. Integration of abscisic acid signalling into plant responses. *Plant. Biol.,* 8(3): 314-325.

Deikman, J., M. Petracek and J.E. Heard. 2012. Drought tolerance through biotechnology: improving translation from the laboratory to farmers' fields. *Curr. Opin. Biotechnol*., 23(2): 243-250.

Do, T.Q., A.Y. Hsu, T. Jonassen, P.T. Lee and C.F. Clarke. 2001. A defect in coenzyme Q biosynthesis is responsible for the respiratory deficiency in *Saccharomyces cerevisiae* abc1 mutants. *J. Biol. Chem.,* 276(21): 18161-18168.

Fujita, Y., K. Nakashima, T. Yoshida, T. Katagiri, S. Kidokoro, N. Kanamori, T. Umezawa, M. Fujita, K. Maruyama and K. Ishiyama. 2009. Three SnRK2 protein kinases are the main positive regulators of abscisic acid signaling in response to water stress in *Arabidopsis*. *Plant. Cell. Physiol.,* 50(12): 2123-2132.

Fukao, T. and L. Xiong. 2013. Genetic mechanisms conferring adaptation to submergence and drought in rice: simple or complex? *Curr. Opin. Plant. Biol.,* 16(2): 196-204.

Fukao, T., E. Yeung and J. Bailey-Serres. 2011. The submergence tolerance regulator SUB1A mediates crosstalk between submergence and drought tolerance in rice. *Plant. Cell.,* 23(1): 412-427.

Gao, Q.S., Z. Yang, Y. Zhou, Z.T. Yin, J. Qiu, G.H. Liang and C.W. Xu. 2012. Characterization of an ABC1 kinase family gene *OsABC1-2* conferring enhanced tolerance to dark-induced stress in rice. *Acta . Agronomica. Sinica* .,498(2): 155-163.

Gao, Q.S., Z.F. Yang, Y. Zhou, D. Zhang, C.H. Yan, G.H. Liang and C.W. Xu. 2010. Cloning of an ABC1-like gene *ZmABC1-10* and its responses to cadmium and other abiotic stresses in maize (*Zea mays* L.). *Gene,* 36(12): 2073-2083.

Gao, Q.S., D. Zhang, X. Liang and C.W. Xu. 2011. Systematic identification of rice ABC1 gene family and its response to abiotic stress. *Rice. Sci.,* 18(3): 167-177.

Hadiarto, T. and L. S.P. Tran. 2011. Progress studies of drought-responsive genes in rice. *Plant. Cell. Rep.,* 30(3): 297-310.

Hong, Z., K. Lakkineni, Z. Zhang and D.P.S. Verma. 2000. Removal of feedback inhibition of Δ1-pyrroline-5-carboxylate synthetase results in increased proline accumulation and protection of plants from osmotic stress. *Plant. Physiol.,* 122(4): 1129-1136.

Hsieh, E.J., J.B. Dinoso and C.F. Clarke. 2004. A $tRNA^{TRP}$ gene mediates the suppression of *cbs2-223* previously attributed to *ABC1/COQ8*. *Biochem. Biophys. Res. Commun.*,317(2): 648-653.

Jasinski, M., D. Sudre, G. Schansker, M. Schellenberg, S. Constant, E. Martinoia and L. Bovet. 2008. AtOSA1, a member of the Abc1-like family, as a new factor in cadmium and oxidative stress response. *Plant. Physiol.,* 147(2): 719-731.

Joo, J., Y.H. Lee and S.I. Song. 2014. Overexpression of the rice basic leucine zipper transcription factor OsbZIP12 confers drought tolerance to rice and makes seedlings hypersensitive to ABA. *Plant. Biotechnol. Rep.,* 8(6): 431-441.

Ke, Y.G., Z.J. Yang, S.W. Yu, T.F. Li, J.H. Wu, H. Gao, Y.P. Fu and L.J. Luo. 2014. Characterization of OsDREB6 responsive to osmotic and cold stresses in rice. *J. Plant. Biol.,* 57(3): 150-161.

Kobayashi, Y., M. Murata, H. Minami, S. Yamamoto, Y. Kagaya, T. Hobo, A. Yamamoto and T. Hattori. 2005. Abscisic acid-activated SNRK2 protein kinases function in the gene-regulation pathway of ABA signal transduction by phosphorylating ABA response element-binding factors. *Plant. J.,* 44(6): 939-949.

Leonard, C.J., L. Aravind and E.V. Koonin. 1998. Novel families of putative protein kinases in bacteria and archaea: evolution of the "eukaryotic" protein kinase superfamily. *Genome. Res.,* 8(10): 1038-1047.

Livak, K.J. and T.D. Schmittgen. 2001. Analysis of relative gene expression data using Real-Time quantitative PCR and the $2^{\Delta\Delta\text{-Ct}}$ Method. *Methods*, 25(4): 402-408.

Lu, G., C. Gao, X. Zheng and B. Han. 2009. Identification of OsbZIP72 as a positive regulator of ABA response and drought tolerance in rice. *Planta,* 229(3): 605-615.

Lundquist, P.K., J.I. Davis and K.J. van Wijk. 2012. ABC1K atypical kinases in plants: filling the organellar kinase void. *Trends. Plant. Sci.,* 17(9): 546-555.

Lundquist, P.K., A. Poliakov, L. Giacomelli, G. Friso, M. Appel, R.P. McQuinn, S.B. Krasnoff, E. Rowland, L. Ponnala and Q. Sun. 2013. Loss of plastoglobule kinases ABC1K1 and ABC1K3 causes conditional degreening, modified prenyl-lipids, and recruitment of the jasmonic acid pathway. *Plant Cell*, 25(5): 1818-1839.

Luo, A.D., L. Liu, Z.S. Tang, X.Q. Bai, S.Y. Cao and C.C. Cai. 2005. Down-regulation of *OsGRF1* gene in rice *rhd1* mutant results in reduced heading date. *J. Integr. Plant. Biol.*, 47(6): 745-752.

Martinis, J., G. Glauser, S. Valimareanu and F. Kessler. 2013. A chloroplast ABC1-like kinase regulates vitamin E metabolism in *Arabidopsis*. *Plant. Physiol.,* 162(2): 652-662.

Mishra, G., W.H. Zhang, F. Deng, J. Zhao and X.M. Wang. 2006. A bifurcating pathway directs abscisic acid effects on stomatal closure and opening in *Arabidopsis*. *Science,* 312(5771): 264-266.

Moons, A., A. De Keyser and M. Van Montagu. 1997. A group 3 LEA cDNA of rice, responsive to abscisic acid, but not to jasmonic acid, shows variety-specific differences in salt stress response. *Gene*, 191(2): 197-204.

Nakashima, K., Y. Ito and K. Yamaguchi-Shinozaki. 2009. Transcriptional regulatory networks in response to abiotic stresses in *Arabidopsis* and grasses. *Plant. Physiol.,* 149(1): 88-95.

Ning, J., X.H. Li, L.M. Hicks and L.Z. Xiong. 2010. A Raf-like MAPKKK gene *DSM1* mediates drought resistance through reactive oxygen species scavenging in rice. *Plant. Physiol.,* 152(2): 876-890.

Ouyang, S.Q., Y.F. Liu, P. Liu, G. Lei, S.J. He, B. Ma, W.K. Zhang, J.S. Zhang and S.Y. Chen. 2010. Receptor-like kinase OsSIK1 improves drought and salt stress tolerance in rice (*Oryza sativa*) plants. *Plant. J.,* 62(2): 316-329.

Park, G.G., J.J. Park, J. Yoon, S.N. Yu and G. An. 2010. A RING finger E3 ligase gene, *Oryza sativa* Delayed Seed Germination 1 (*OsDSG1*), controls seed germination and stress responses in rice. *Plant. Mol. Biol.,* 74(4-5): 467-478.

Rohila, J.S. and Y. Yang. 2007. Rice mitogen-activated protein kinase gene family and its role in biotic and abiotic stress response. *J. Integr. Plant. Biol.,* 49(6): 751-759.

Tamura, K., D. Peterson, N. Peterson, G. Stecher, M. Nei and S. Kumar. 2011. MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Mol. Biol. Evol.,* 28(10): 2731-2739.

Tang, N., H. Zhang, X.H. Li, J.H. Xiao and L.Z. Xiong. 2012. Constitutive activation of transcription factor OsbZIP46 improves drought tolerance in rice. *Plant. Physiol.,*158(4): 1755-1768.

Todaka, D., K. Nakashima, K. Shinozaki and K. Yamaguchi-Shinozaki. 2012. Toward understanding transcriptional regulatory networks in abiotic stress responses and tolerance in rice. *Rice,* 5(1): 1-9.

Umezawa, T., M. Fujita, Y. Fujita, K. Yamaguchi-Shinozaki and K. Shinozaki. 2006. Engineering drought tolerance in plants: discovering and tailoring genes to unlock the future. *Curr. Opin. Biotechnol.,* 17(2): 113-122.

Wang, C.X., R.L. Jing, X.G. Mao, X.P. Chang and A. Li. 2011. TaABC1, a member of the activity of bc1 complex protein kinase family from common wheat, confers enhanced tolerance to abiotic stresses in *Arabidopsis*. *J. Exp. Bot.,* 62(3): 1299-1311.

Wilkinson, S. and W.J. Davies. 2002. ABA-based chemical signalling: the co-ordination of responses to stress in plants. *Plant. Cell. Environ.,* 25(2): 195-210.

Xiang, Y., N. Tang, H. Du, H.Y. Ye and L.Z. Xiong. 2008. Characterization of OsbZIP23 as a key player of the basic leucine zipper transcription factor family for conferring abscisic acid sensitivity and salinity and drought tolerance in rice. *Plant. Physiol.,*148(4): 1938-1952.

Xie, L.X., E.J. Hsieh, S. Watanabe, C.M. Allan, J.Y. Chen, U.C. Tran and C.F. Clarke. 2011. Expression of the human atypical kinase ADCK3 rescues coenzyme Q biosynthesis and phosphorylation of Coq polypeptides in yeast *coq8* mutants. *Biochim. Biophys. Acta.,* 1811(5): 348-360.

Xiong, L.M., K.S. Schumaker and J.K. Zhu. 2002. Cell signaling during cold, drought, and salt stress. *Plant Cell,* 14(suppl 1): S165-S183.

Yamaguchi-Shinozaki, K., J. Mundy and N.H. Chua. 1990. Four tightly linked *rab* genes are differentially expressed in rice. *Plant. Mol. Biol.,* 14(1): 29-39.

Yang, S.G., T. Li, M. Liu, S. Gao, S. Yang, L. Li and C.W. Yang. 2012a. Phylogenetic, structure and expression analysis of *ABC1Ps* gene family in rice. *Biologia. Plantarum,* 56(4): 667-674.

Yang, S.G., X.Q. Zeng, T. Li, M. Liu, S.C. Zhang, S. Gao, Y.Q. Wang, C.L. Peng, L. Li and C.W. Yang. 2012b. *AtACDO1*, an ABC1-like kinase gene, is involved in chlorophyll degradation and the response to photooxidative stress in *Arabidopsis*. *J. Exp. Bot,* 72(63):3959-3973.

Yang, S.Y., Q. Zhang, T. Li, J.J. Du, S.G. Yang and C.W. Yang. 2012. AtSIA1, an ABC1-like kinase, regulates salt response in *Arabidopsis*. *Biologia*, 67(6): 1107-1111.

Zhang, S., I. Haider, W. Kohlen, L. Jiang, H. Bouwmeester, A.H. Meijer, H. Schluepmann, C.M. Liu and P.B. Ouwerkerk. 2012. Function of the HD-Zip I gene *Oshox22* in ABA-mediated drought and salt tolerances in rice. *Plant. Mol. Biol*., 80(6): 571-585.

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