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Full Length Research Paper

Overexpression of *BrSAC1* encoding a phosphoinositide phosphatase isolated from Chinese cabbage (*Brassica rapa* L) improved tolerance to cold, dehydration, and salt stresses in transgenic tobacco

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This study demonstrates the isolation and characterization of cDNA encoding a phosphoinositide phosphatase (PIP) from a stem cell cDNA library of Chinese cabbage *(Brassica rapa*) seedling. The full length gene (*BrSAC1*; GenBank accession no., GU434275) contained 1999 base pairs (bp), with an open reading frame of 1785 bp, encoding a polypeptide of 594 amino acids with a predicted molecular weight of 65 kDa, including a putative N-terminal signal peptide (the signal peptide counted within the 594 residues). Other regions found within the sequence include a conserved KXKXX COPI-binding motif and a consensus Cx5R (T/S) catalytic motif. BrSAC1 protein shares 92% identity with AtSac1B, and 86% identity with AtRHD4 at the amino acid level. Gene expression analyses revealed that *BrsSAC1* was constitutively expressed at high levels in the pistil, stamen and flower bud, whereas it was expressed at low levels in the leaf and stem. In addition to injury, *BrSAC1* expression was also induced in response to different types of stress condition, namely cold, desiccation, salt, submergence, abscisic acid and heavy metals. Overexpression of *BrSAC1* in transgenic tobacco conferred tolerance to cold, dehydration, and salt stress at the seed germination/seedling stage as reflected by the percentage of germination/green seedlings, the fresh weight of seedlings and their development pattern. Our data suggest that *BrSAC1* is an important stress response determinant in plants.

Key words: Abiotic stress, *Brassica rapa*, phosphoinositide phosphatase, transgenic plant.

INTRODUCTION

Plant growth is strongly affected by a variety of major obstacles affecting plant growth and crop environmental stresses. Cold, salt, and drought are the productivity. In response to these stresses, plants initiate signaling cascades leading to the development of defense responses. Phosphoinositides (PIs) are signaling molecules that regulate cellular events, including vesicle targeting and interactions between membrane and cytoskeleton. To date, various enzymes involved in PI

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signaling and biosynthesis have been identified and characterized in animals (Martin, 1998; De Matteis et al., 2002). Compared to animals, in plants the role of PIs is still poorly understood. During the last decade, however, new information has emerged showing involvement of PIs in various plant processes, such as calcium mobilization, signal transduction, cytoskeletal rearrangements and membrane trafficking (Wang, 2004) in addition to the defense against attack by pathogens (Laxalt and Munnik, 2002). Recent research indicates that PIs play important roles in development, including stress signaling (Meijer et al., 2001; Xiong et al., 2001), growth (Williams et al., 2005), and vascular patterning (Carland and Nelson, 2004). PI phosphatases (PIP) are classified into three main groups, 3-, 4-, or 5-phosphatases on the basis of the position of the phosphate-inositol head group being hydrolyzed (Takenawa and Itoh, 2001). The group of phosphatases called suppressor of actin (SAC) domain phosphatases have been identified in yeast and animal; these phosphatases were shown to hydrolyze the phosphates on multiple positions on the inositol head group of PIs (Hughes et al., 2000). As in other eukaryotic systems, PI metabolism in higher plants is considered important in signal transduction. The involvement of phospholipid signaling in the cell responses to stress stimuli, including salt, osmotic, temperature, and pathogen stresses, has been shown, and multiple members of the PI pathway are thought to mediate stress responses through different mechanisms.

In this study, we isolated the *BrSAC1* gene encoding a PIP from the cDNA of *Brassica rapa* seedling and characterized its modulation of PI signals during abiotic stress response. Also, introgression of *BrSAC1* into tobacco led to stress tolerance when assessed at the seedling stage.

MATERIALS AND METHODS

Plant materials and stress treatments

Chinese cabbage (*B. rapa* var. OSOME) was aseptically grown on Murashige Skoog (MS) agar medium (Murashige and Skoog, 1962) in a culture room under a 16:8 h light:dark period at 25°C. Salt and hormone treatments were conducted, after nine days of growth, on seedlings that were transferred to fresh liquid half-strength MS medium without sucrose (MSH medium) containing either 250 mM NaCl or 100 µM abscisic acid (ABA) for 24 h. Induction of cold stress was examined on seedlings grown in the above conditions for 24 h after transferring to 4°C. Membrane fluidity treatments using chemical modulators were conducted on cut leaves of seedlings exposed to 5, 10, and 15 mM benzyl alcohol at 25°C for 3 h followed by growth at 8°C for 24 h. Cut leaves of seedlings were kept at 25°C for 6 h in the presence of 2, 4 and 6% dimethyl sulfoxide (DMSO), while the control leaves were kept in water at 25°C.

Isolation of full-length cDNA of *BrSAC1*

A partial *BrSAC1* sequence was identified in the dbEST division of GenBank and searches were conducted using basic local alignment search tool (BLAST) with a specific SAC domain motif as the query sequence. The full-length cDNA was isolated by RACE technology using the SMART RACE-PCR kit (Clontech, Palo Alto, CA, USA) (Lee et al., 2009).

RNA isolation and real-time PCR

Total RNA from leaf tissues was extracted using Trizol reagent (Invitrogen, USA) according to the manufacturer's protocol. The specific primers for the analysis of *BrSAC1* expression in germinating seeds were: *BrSAC1*F3 5′-CGGAAAGAGTTGCATTCAGC-3′ and *BrSAC1*R3 5′-ATGATGGCTGGAATGCCCT-3′. Real-time polymerase chain reaction (qRT-PCR) was performed using a Bio-RAD I Cycler IQ5 machine as previously described (Ali-Benali et al., 2005) using RT pre-mix (TOYOBO Co., Japan). The mean threshold cycle (Ct) values of triplicate PCR reactions were computed and relative quantification of expression levels was performed using the comparative Ct method (Livak and Schmittgen, 2001). The fold change in total RNA of the target gene relative to the reference gene (actin) was determined by the following formula: fold change = $2^{-\Delta\Delta Ct}$, where $\Delta\Delta Ct$ = ($Ct_{target\ gene}$ - $Ct_{actin\ gene}$) transgenic plants - ($Ct_{target\ gene}$ - $Ct_{actin\ gene}$) wild-type plants.

First-strand cDNA was generated using Superscript™ III reverse transcriptase according to manufacturer's instructions (Invitrogen, USA). BrSAC1 was amplified using 1 ul cDNA template added with the primer pairs *BrSAC1*F2 5′-ATGGAGGCTCCTAAGCA-3′ and *BrSAC1*R2 5′-CGCAAAGAGTTGCATTCAGC-3′. The PCR amplification program consisted of an initial step at 94°C for 5 min followed by 30 cycles of 94°C for 1 min, 58°C for 1 min, 72°C for 2 min, and a final step at 72°C for 10 min.

Generation of *BrSAC1* transgenic tobacco plants

Full-length sequence of *BrSAC1* amplified from a pGEM-T easy vector with the primers *BrSAC1*F1 (5′-ATGGAGGCTCCTCCTAAGCACA-3′) and *BrSAC1*R1 (5′-TCAAGGTCTGGGCTTGTGCAG-3′) was confirmed by sequencing and inserted into the *Sfi*I site of a pBig plant binary vector system for overexpression; the vector also contained a modified cauliflower mosaic virus (CaMV) 35S promoter. The pBig plant binary vector containing complete coding sequence of *BrSAC1* was introduced into tobacco seedlings via *Agrobacterium tumefaciens*-mediated transformation according to the procedure of Horsch et al. (1985).

Four-week-old T2 generation tobacco seedlings were analysed for the presence of the transgene. DNA was extracted from the leaves of the tobacco seedlings by using cetyltrimethyl ammonium bromide (CTAB) method (Rogers and Bendich, 1994). The primers used for PCR amplification were *BrSAC1*F2 and *BrSAC1*R2, with reaction conditions as described above.

Analysis of transgenic plants for abiotic stress tolerance

Four homozygous T2 generation transgenic tobacco (*Nicotiana tabacum* cv. Samson) plants were used in all subsequent abiotic stress assays. The seeds of transgenic and non-transgenic (wild type, WT) plants were germinated and grown on MS medium for 16 days in a culture room under a 16:8 h light:dark period at 24°C.

For salt, cold, and dehydration stress treatments, (Figure 4), the seedlings were germinated and grown according to the above procedure for 14 days and then transferred to ½ MS media containing 250 mM NaCl, 4°C cold, and 0.3 M and 0.4 M mannitol. After 14 days, the fresh weight (FW) and shoot and root lengths were measured. Statistical analyses were performed using a Student's *t* test in Microsoft Excel. P-values below 0.05 were consi-

dered significant. To determine the effects of salt, cold, and dehydration stress on transgenic tobacco plants (Figures 5 to 7), seeds of WT and transgenic plants were germinated and grown on ½ MS medium for various times (noted below) in a culture room according to the procedure described above. The effect of salt stress resulting in chlorosis was examined in WT and transgenic seedlings (21 days) that had been transferred to sterile tissue paper soaked with a 250 mM NaCl MSH solution for four days. After treatment, seedlings were washed briefly in sterile Milli-Q water and allowed to grow and recover for eight days on MSH in a culture room, and then the number of green seedlings was determined. Four leaf discs of transgenic (lines 2 and 4) and WT plants (21 days) were floated in 250 mM NaCl MSH solution treatment versus no salt control MSH solution for four days to examine status and chlorophyll content. The leaf disk chlorophyll content (mg/g FW) was measured using the method described by Aono et al. (1993) and was shown as mean ± standard deviation. To examine the effects of cold stress treatment, transgenic and WT plants (21 days) were transferred to a cold chamber (4°C) for 15 days. After treatment, the plants were transferred to culture room conditions and allowed to grow and recover for 15 days. Individual plant FW (mg) was measured and is shown as the mean ± standard deviation. To examine the effects of dehydration stress on germinating seeds of transgenic and WT plants, the seeds were germinated on 0.3 and 0.4 M mannitol and observations (percent germination, FW) of representative plants were recorded over a period of eight days according to the method of Mukhopadhyay et al. (2004).

Multiple sequence alignment and phylogenetic analysis

Multiple sequence alignment of the *BrSAC1* gene was performed using Clustal X version 1.83 (Thompson et al., 1997). A phylogenetic tree was constructed using DNA Data Bank of Japan (DDBJ; http://clustalw.ddbj.nig.ac.jp/top-e.html), and bootstrap analysis was performed with 1,000 samplings.

RESULTS

Cloning and sequence analysis of *BrSAC1*

A partial cDNA of *BrSAC1* was identified by a database search of the dbEST division of GenBank with a specific SAC domain motif as the query sequence. The full-length cDNA of BrSAC1 was isolated using rapid amplification of cDNA ends-polymerase chain reaction (RACE-PCR), revealing the gene to be 1,999 bp in length and consisting of a 114 bp 5′-untranslated region (UTR), a complete opening reading frame (ORF) of 1,785 bp encoding a polypeptide of 594 amino acids (65 kDa) and a 3′-UTR of 100 bp (data not shown).

Sequence BLAST analysis showed that the BrSAC1 protein exhibited identity with AtSac1B (*Arabidopsis thaliana*), NP201403 (92%); AtRHD4 (*A. thaliana*), NP190714 (86%); SAC1 (*Saccharomyces cerevisiae*), CAA82057 (38%); Sac1 (*Drosophila melanogaster*), NM138243 (37%); Rat Sac1 (*Rattus norvegicus*), NP446250 (35%); mouse Sac1 (*Mus musculus*), NM030692 (35%); human Sac1 (*Homo sapiens*), NM014016 (35%); rice Sac1 (*Oryza sativa*), NP001067755 (54%); and maize Sac1 (*Zea mays*), BT086272 (48%). A comparative analysis of the *BrSAC1* gene sequence with known SAC domain containing sequences revealed the presence of a KXKXX conserved COPI binding motif and the consensus CX5R(T/S) sequence for the Sac1-catalytic domain (Figure 1A).

Further, both BrSAC1 and AtSac1B contain N-terminal KXKXX motifs that serve as binding sites for the coatomer (COPI) complex. This COPI interaction motif is considered a unique feature of mammalian Sac1s, as it is not conserved in other plants, yeast, or Drosophila Sac1 proteins (Figure 1B). The phylogenetic relationship of the deduced amino acid sequence of *BrSAC1* to the SAC domains from other organisms was determined using Clustal W within the BioEdit program (Figure 1C).

Expression of the *BrSAC1* gene in various organs and during seed germination

Expression analysis of the *BrSAC1* gene transcript was performed in various vegetative tissues (leaf, stem and root), reproductive tissues (stamen, pistil, petal, flower bud, calyx of flower, and flower stalk), and during seed germination. Semi-quantitative reverse transcription (RT)-PCR analysis showed varying expression of *BrSAC1* gene across tissues, highest in the stamen among reproductive tissues and in leaf among vegetative tissues (Figure 2A). An examination of *BrSAC1* expression during the first 72 h of seed germination revealed an increase in mRNA levels of *BrSAC1* that peaked at 36 h, about 20 fold higher than that at 0 h, with a fast decrease at subsequent times examined (Figure 2B).

Expression pattern of *BrSAC1* under different abiotic stresses

The results of qRT-PCR analysis showed that *BrSAC1* was induced under various abiotic stresses. *BrSAC1* transcripts showed rapid induction (9.0 fold) peaking at 1 to 2 h after exposure to cold (4°C) conditions, followed by a gradual decrease in expression levels to pre-treatment levels over the next several hours of exposure. In the presence of salt stress (250 mM NaCl), transcript levels were rapidly induced several fold by 1 h, maintaining a high level of induction after 3 to 8 h, then returning to pre-treatment levels (Figure 3). Expression of *BrSAC1* in response to the stress hormone ABA was also determined and exogenous ABA treatment (100 µM) resulted in strong *BrSAC1* induction within 1 h, gradually decreasing to pre-treatment levels by 8 h. We also wanted to examine the role of membrane fluidity and the cold induction of *BrSAC1* transcript. Membrane rigidification at low temperatures can be prevented by benzyl alcohol (BA) that acts as a membrane fluidizer. Similar examinations can also be simulated at room temperature by treatment with a membrane rigidifier DMSO (Mukhopadhyay et al., 2004). In order to examine

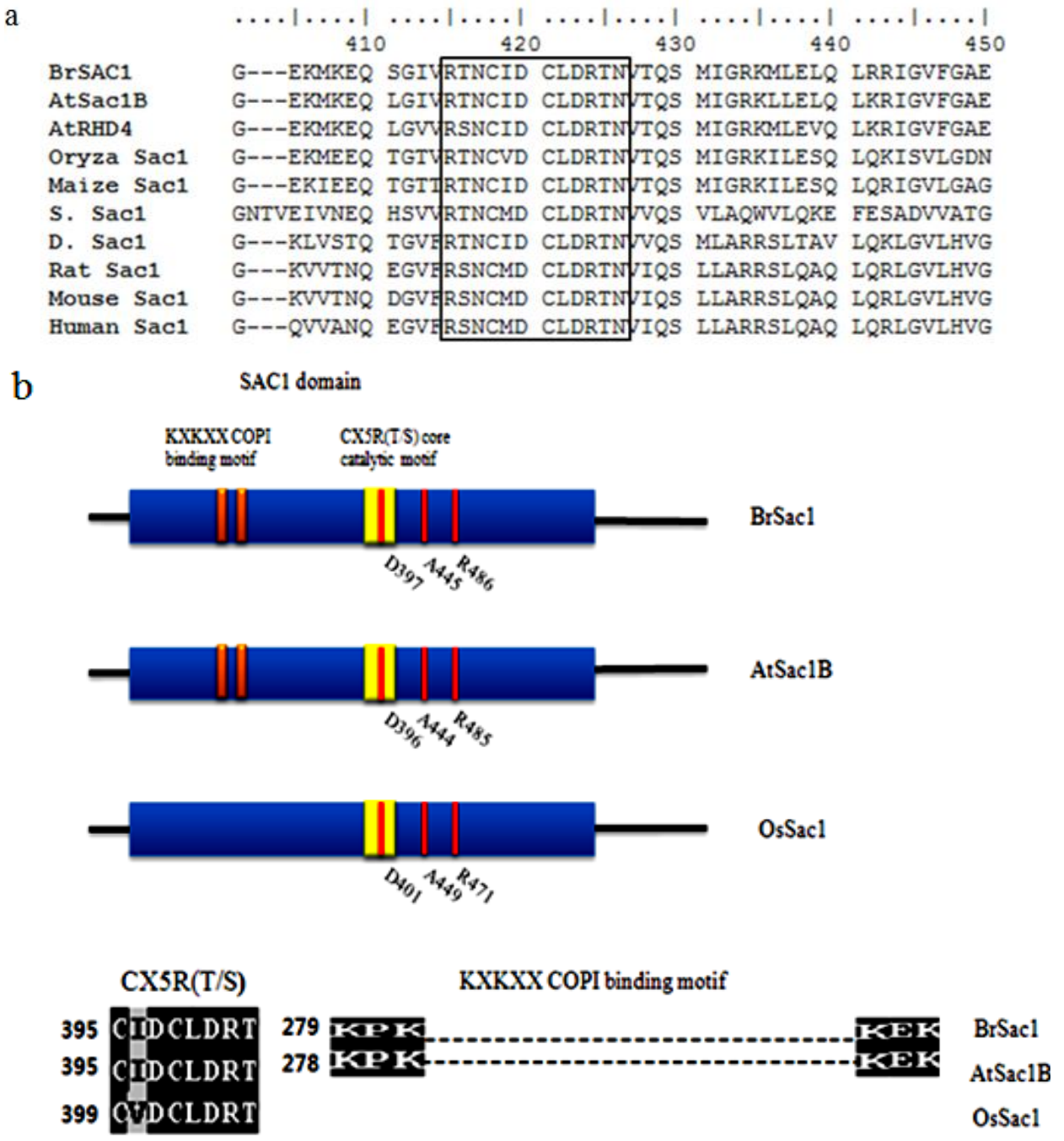
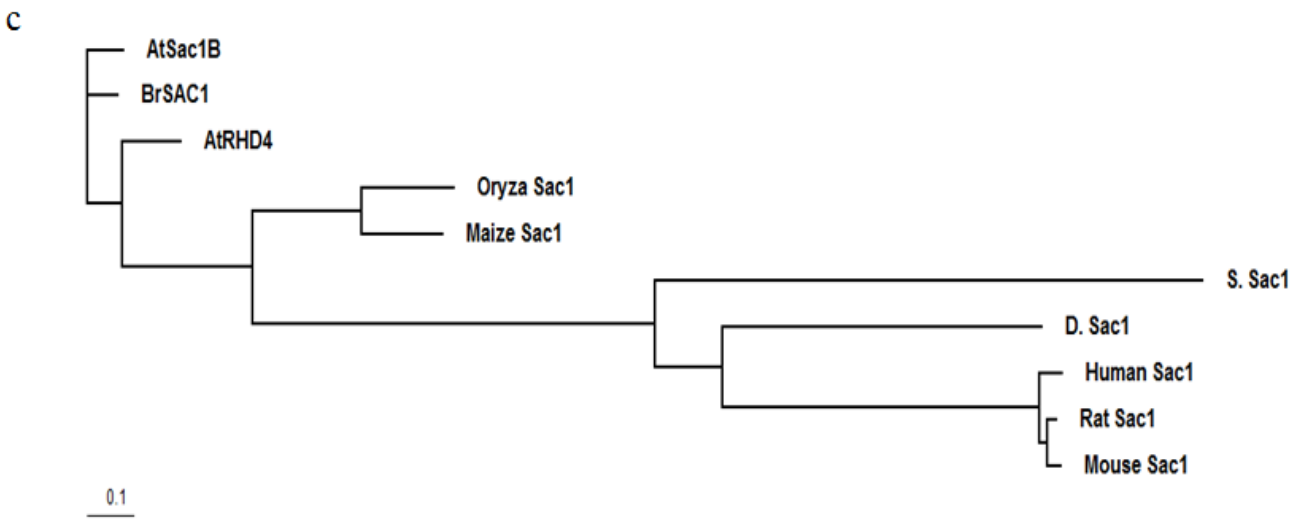


Figure 1. Sequence alignment, domain structure and phylogenetic relationship of *BrSAC1*. **A.** Comparison and deduced amino acid sequence of *BrSAC1* and other Sac1 proteins, AtSac1B (*Arabidopsis thaliana*), NP201403; AtRHD4 (*A. thaliana*), NP190714; SAC1 (*Saccharomyces cerevisiae*), CAA82057; Sac1 (*Drosophila melanogaster*), NM138243; rat Sac1 (*Rattus norvegicus*), NP446250; mouse Sac1 (*Mus musculus*), NM030692; human Sac1 (*Homo sapiens*), NM014016; rice Sac1 (*Oryza sativa*), NP001067755; and Maize Sac1 (*Zea mays*), BT086272. Sequence alignment of the core catalytic domains and the KXKXX COPI binding motifs of the SAC1 protein family. The $CX_5R(T/S)$ core catalytic domain of Sac1 is highly conserved in *yeast*, higher plants, *Drosophila*, and *mammals.* Identical and conserved residues in all aligned sequences are indicated by the rectangle box. **B.** (a) Diagrams of the domain organization of Sac1 proteins. Domains and motifs of interest are highlighted by colored boxes. Blue boxes show the Sac1-catalytic domain, yellow boxes show the CX5R(T/S) core catalytic motif, and orange boxes show the KXKXX COPI binding motif. Three key amino acids within the Sac1 domain known to affect catalytic activity (Rivas et al., 1999; Liu et al., 2008) are indicated in red. (b) Sequence alignment of the core catalytic domains and the KXKXX COPI binding motifs of the Sac1 protein families. Identical amino acids are highlighted by black boxes. **C.** The phylogenetic tree of the Sac1 protein family. *BrSAC1* isolated in this study. A phylogenetic tree was constructed using CLC Main workbench 5 with CLUSTAL W alignments as input using the neighbor-joining method (Saitou and Nei 1987) to construct the tree.



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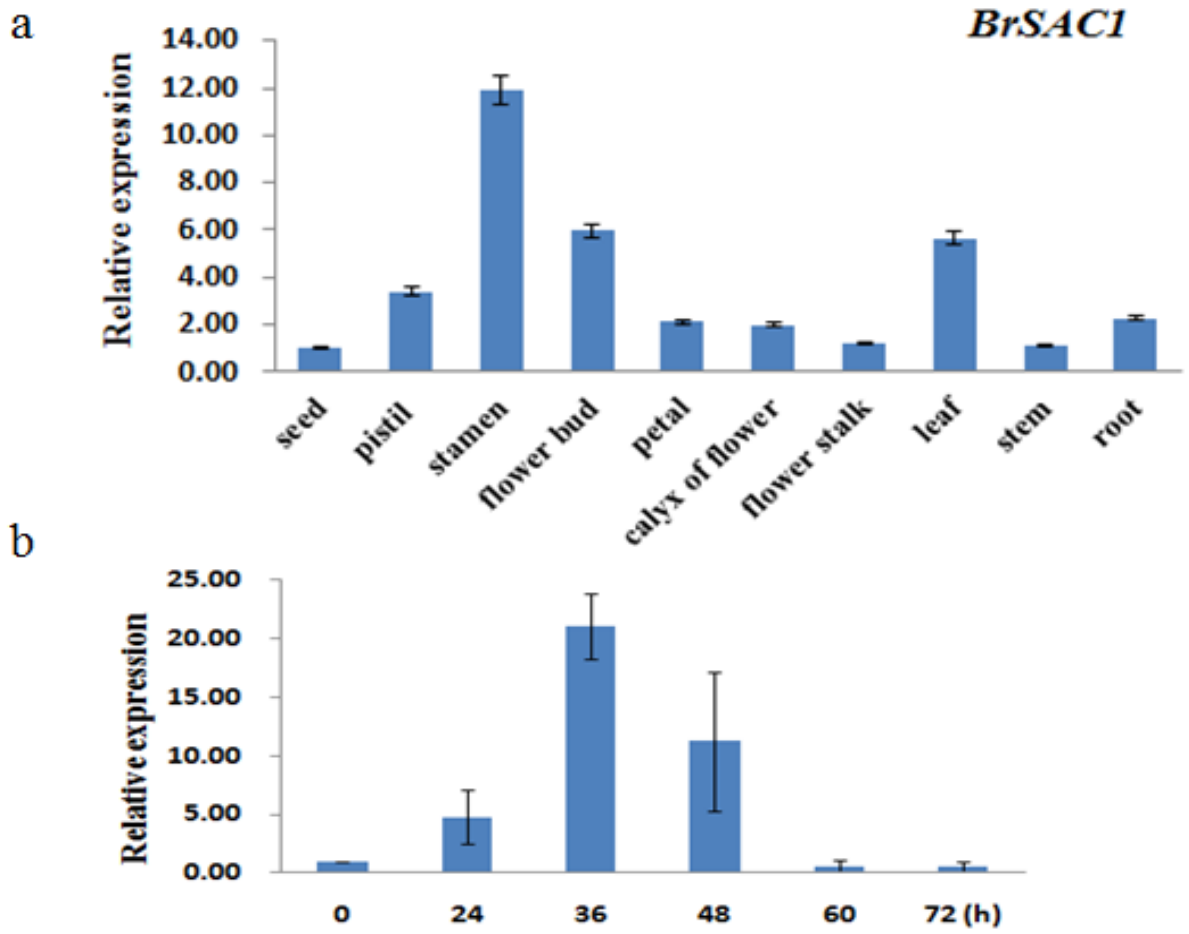
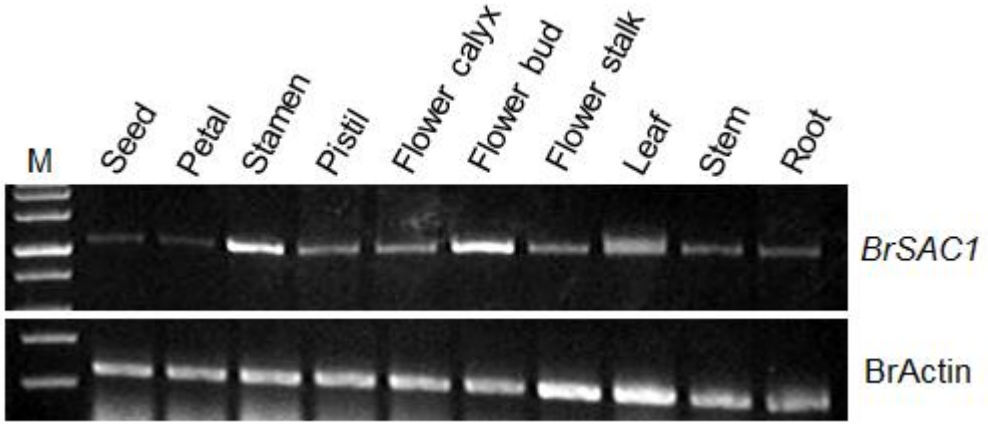


Figure 2. *BrSAC1* showing the broad expression across plant tissues using real-time PCR analysis. **A.** Expression levels of *BrSAC1* were measured in various tissues collected from *Brassica rapa.* **B.** Real-time PCR analysis of *BrSAC1* in germinating seedlings (0-72 h after germination). Amounts of template were calibrated using the mRNA as a reference. Data are presented as means ± standard deviation of three independently replicated experiments.



Supplemental Figure 1 for Figure 2. Han et al. *BrSAC1* showing broad expression across plant tissues. Semi-quantitative reverse transcription-polymerase chain reaction (RT-PCR) analysis of *BrRZFP1* was conducted in various tissues collected from *Brassica rapa.* Actin was used as a positive control and was detected in all tissues shown as the set of lower bands.

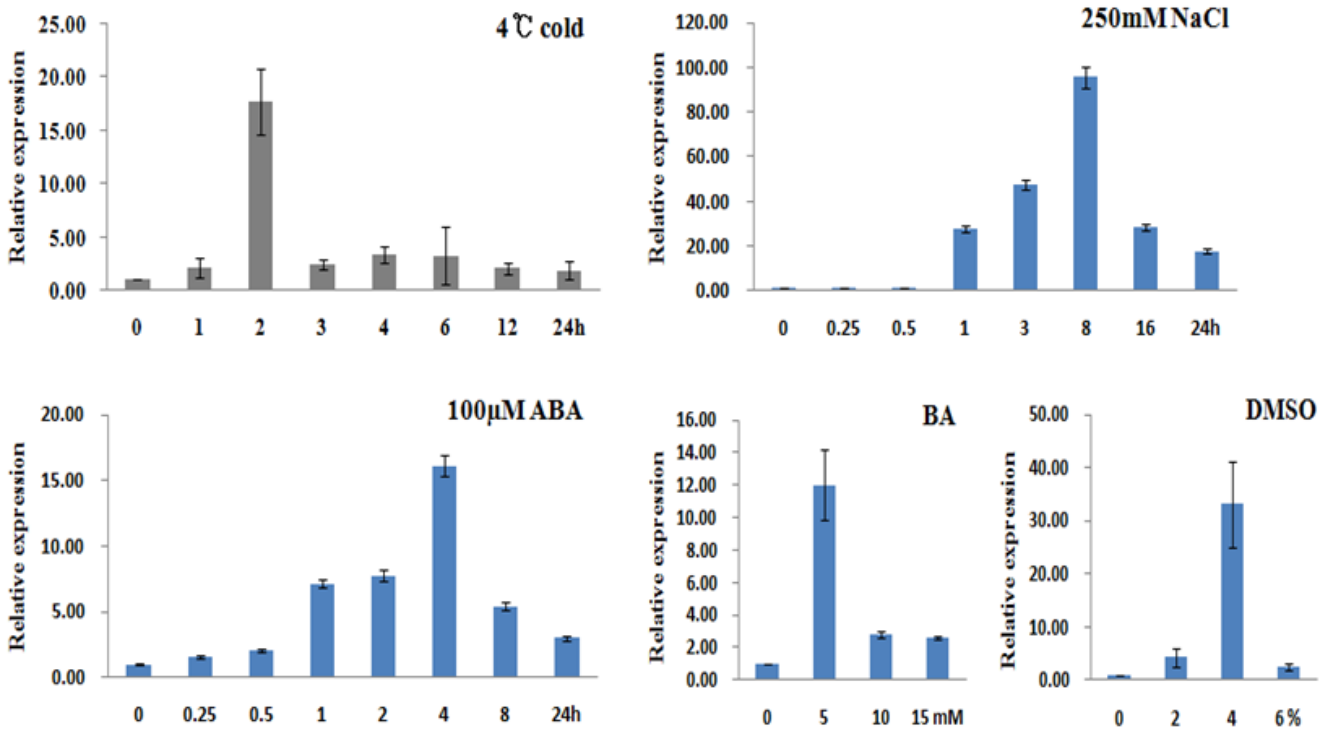


Figure 3. *BrSAC1* expression is induced by abiotic stress treatments in *Brassica* seedlings. Expression levels were examined by RT-PCR at various times after treatment with cold (4°C), salt (250 mM NaCl), or 100 µM abscisic acid (ABA). Expression profiles were also examined in the presence of various concentrations of chemical modulators of membrane fluidity benzyl alcohol (BA) at 4°C and DMSO at room temperature. Actin was used as a positive control and to determine relative expression for each treatment.

the role of membrane fluidity in the cold induction of the *BrSAC1* gene, plants were examined either after cold treatment for 3 h in the presence of BA or at room temperature for 6 h in the presence of DMSO. The cold-induced accumulation of the *BrSAC1* gene was reduced by the inhibition of membrane rigidification by increasing concentrations of BA. Additionally, *BrSAC1* expression declined after treatment of seedlings with elevated concen-

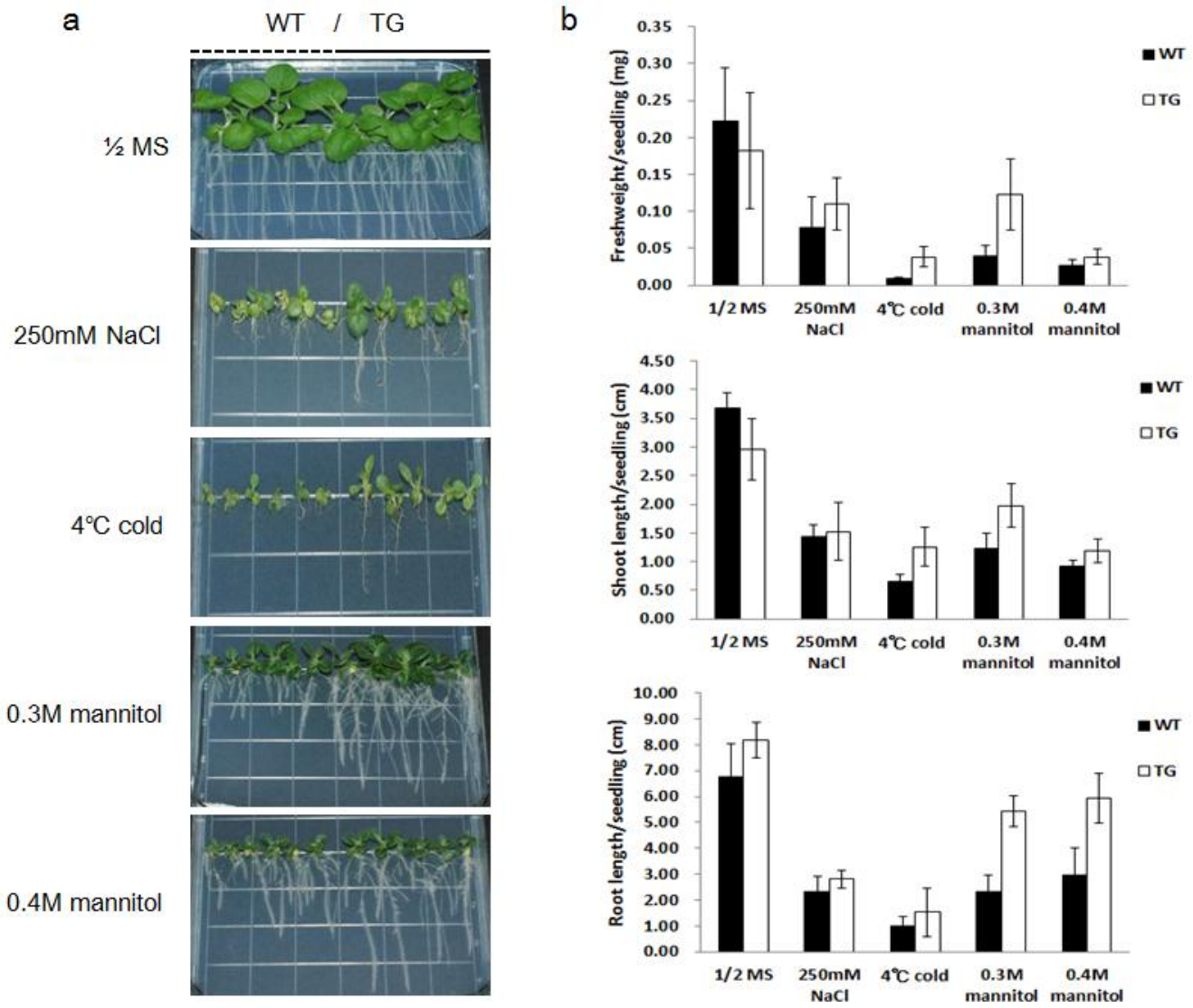


Figure 4. Plants overexpressing *BrSAC1* showing enhanced tolerance to stress. Tobacco plants overexpressing *BrSAC1* (transgenic lines: TG) and WT plants were examined under various stress conditions. The 10-day-old seedlings grown on ½ MS medium were transferred to new media containing 250 mM NaCl for salt stress, 0.3 M or 0.4 M mannitol for dehydration stress, or placed at 4℃ for cold stress. After 18 days of stress treatment, plants were photographed (A) and examined for relative fresh weight and shoot and root lengths. Data are represented as average ± standard deviation for 5 plants from 1 representative experiment (B). In this analysis, significant *P* values of 0.015 to 0.02 (FW), 0.015 to 0.02 (shoot lengths), and 0.02 to 0.025 (root lengths) were obtained between WT and transgenic lines (TG1 and TG2).

trations of the membrane rigidifier, DMSO, greater than 4% (Figure 3). The decline was thought to be caused by the toxic influence of this compound.

BrSAC1 overexpression increases stress tolerance

To study the physiological functions of *BrSAC1*, we generated several transgenic tobacco plant lines that overexpressed *BrSAC1* by fusing its full-length cDNA sequence to the CaMV 35S promoter in the pBig vector (data not shown). Four positive transgenic plant lines were confirmed by genomic PCR, Southern blot, and RT-PCR (data not shown). No significant morphological or growth differences were observed between the transgenic and non-transgenic plants under standard growth conditions. Each of the transgenic lines was examined for responses to salt stress in comparison to WT. Seedlings were stressed for 18 days with salt (250 mM NaCl), cold (4°C), or dehydration (0.3 M and 0.4 M mannitol) stresses, and FW, and shoot and root lengths were examined (Figure 4). After salt stress, all seedlings (WT and TG) showed significant reductions in each of the physiological categories measured. Overexpressing

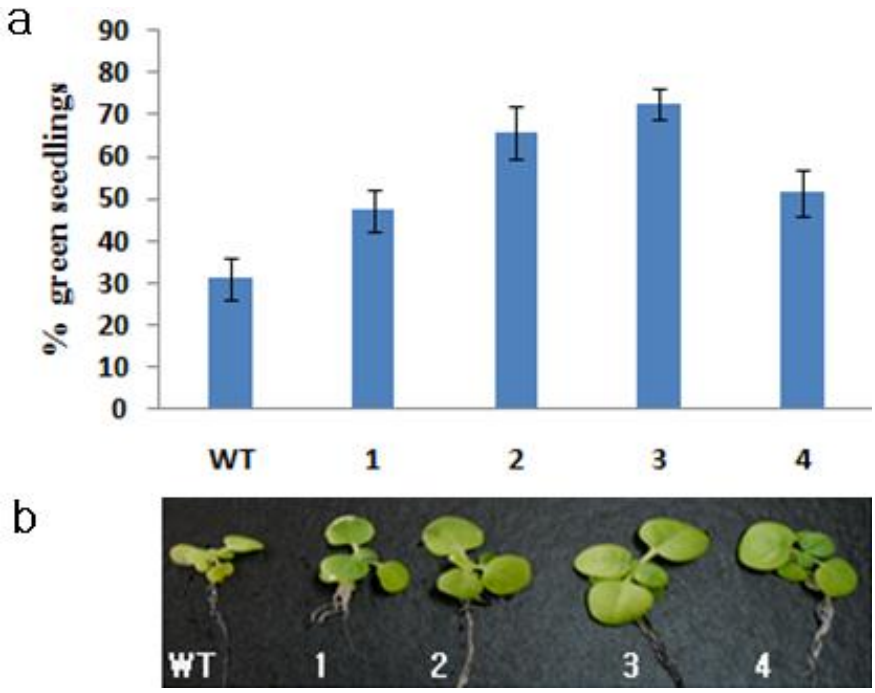


Figure 5. Plants overexpressing *BrSAC1* showed increased salt tolerance. Seedlings of tobacco seedlings from transgenic lines (1 to 4) overexpressing *BrSAC1* were examined for effects of salt treatment. **A.** The number of non-chlorotic (Green) 21 day old seedlings (WT and transgenic lines) were counted following a 4 day 250 mM NaCl salt-stress treatment and eight day recovery period. **B.** Pictures of representative seedlings from (A) are shown at the end of the recovery period.

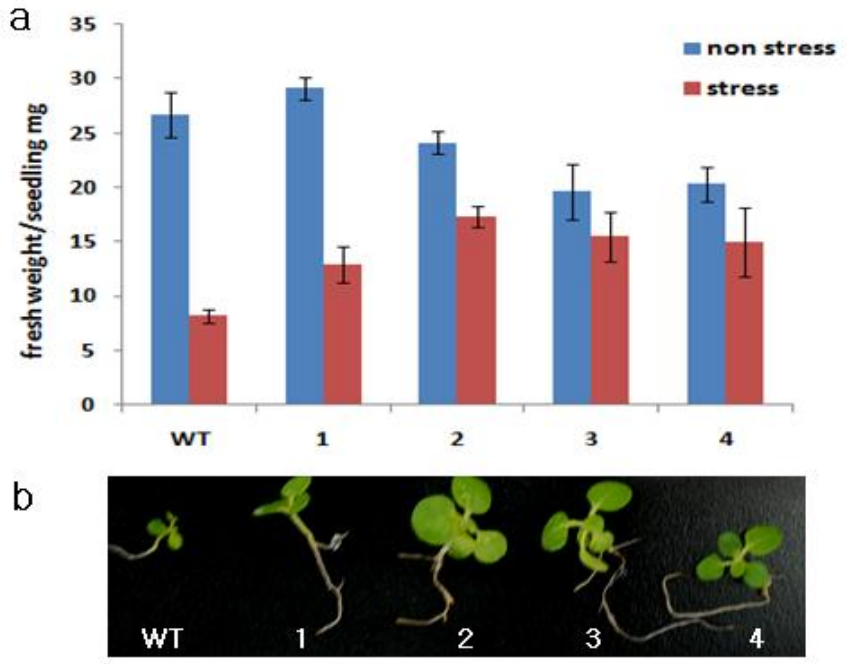


Figure 6. Plants overexpressing *BrSAC1* showing increased cold tolerance. Tobacco seedlings from transgenic lines (1 to 4) overexpressing *BrSAC1* were examined for effects of cold stress: **A.** Fresh weight of 21-day-old seedlings (WT and transgenic lines) that were cold-stressed (4°C) for 15 days, followed by a 15 day recovery, was measured for comparison with that of unstressed controls. **B.** Pictures of representative seedlings from (A) at the end of the recovery period.

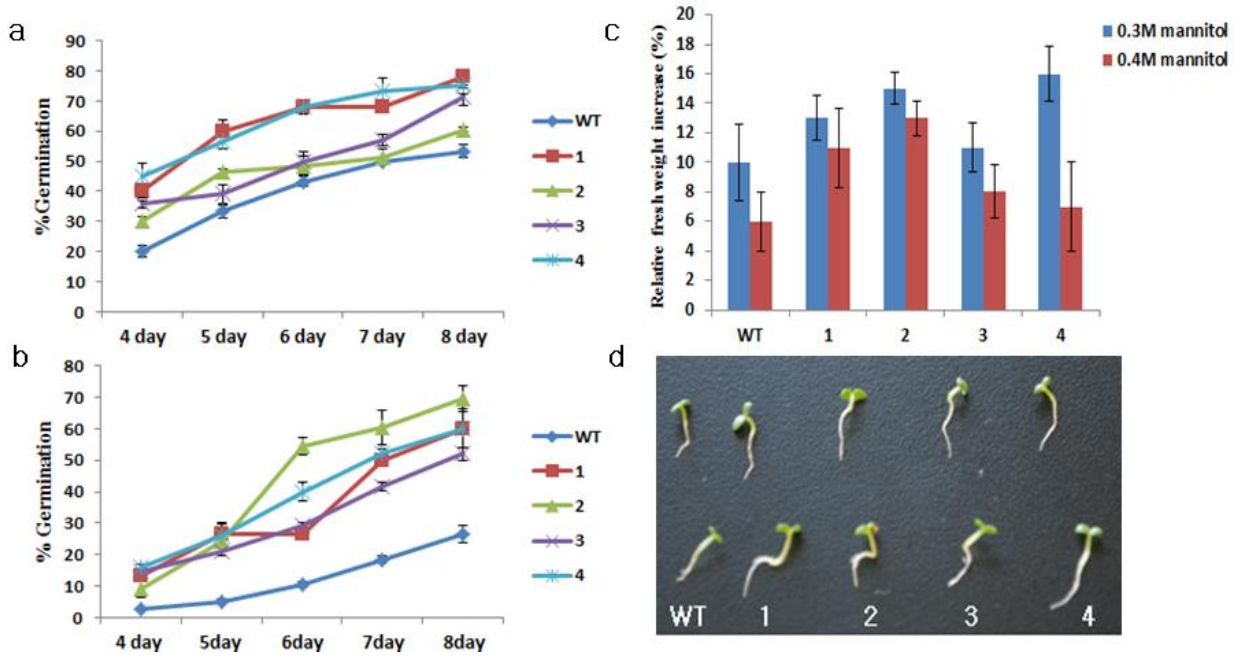


Figure 7. Plants overexpressing *BrSAC1* showing increased dehydration tolerance. Tobacco seedlings overexpressing *BrSAC1* (transgenic lines 1 to 4) were examined for effects of dehydration stress. Percent germination of seeds (WT and transgenic lines) sown on 0.3 M (A) and 0.4 M (B) mannitol is shown for four to eight days after the start of germination. (C) Percent increase in relative fresh weight (FW) of 8-day-old seedlings germinated on 0.3 M or 0.4 M mannitol is shown relative to the FW of unstressed seedlings. (D) Pictures of representative seedlings of WT and transgenic lines taken 8 days after germination on 0.3 M (upper) and 0.4 M (lower) mannitol.

BrSAC1-carrying lines showed greater tolerance to salt stress than WT having significant increases in FW and shoot and root length of almost twice those of WT-levels. Plants were also examined after salt stress followed by a recovery period. Seedlings were subjected to salt stress in 250 mM NaCl for 4 days and then returned to non-stressed conditions for eight days after which the number of plants without any chlorosis (green seedlings) was counted (Figure 5) while about 30% of WT seedlings remained green; at the end of this period several overexpressing lines showed significant increases in the number of green seedlings, some with more than 70% (Figure 5A). Representative examples of surviving plants from this experiment are shown (Figure 5B) (*P* values of 0.015 and 0.02, respectively, by student's *t*-test).

Overexpressing lines were also examined to determine the role of *BrSAC1* during cold stress by growing seedlings at 4°C for 15 days followed by a recovery period of the same time (Figure 6). An increase in FW was noted in some transgenic lines with advantage levels accounted to 50 to 100% of WT FW (Figure 6A) ($P < 0.02$, by student's *t*-test). In addition, transgenic seedlings appeared to be larger and healthier than WT seedlings after cold stress treatment showing recovery marked by emergence and expansion of third and fourth leaves compared to WT seedlings that only produced undeveloped first two leaves (Figure 6B).

In order to examine the role of *BrSAC1* in dehydration stress tolerance, WT and transgenic lines were grown and analyzed in the presence of stressing levels of mannitol over an eight-day period (Figure 7). Germination was dramatically effected in WT plants, with levels reduced to less than 20% by day 4. At the same time point, transgenic *BrSAC1* lines showed almost three fold increases in percent germination in both the lower (0.3 M) and higher (0.4 M) concentrations of mannitol examined (Figures 7A and B). Similar increased levels of percent germination were observed in almost every *BrSAC1* transgenic line over the period examined, often at levels two-fold or greater compared to WT, suggesting an important role for this gene in dehydration stress tolerance. In addition, the FW of transgenic lines stressed by mannitol was also increased in several cases to two-fold above that of WT plants (Figures 7A and B) (*P* values of 0.01 and 0.015, respectively, by student's *t* test).

Together these results suggest that *BrSAC1* plays an important role in several stress related processes, and overexpression of *BrSAC1* may lead to transgenic plants becoming tolerant to salt, cold, and dehydration stresses.

DISCUSSION

The SAC domain was first identified in the yeast (*S.*

cerevisiae) Sac1p PIP protein and subsequently in a number of proteins from yeast and animals. It is approximately 400 amino acids in length and characterized by seven conserved motifs. The Sac1 phosphatases represent a major class of PIP phosphatases and have a signature feature of being integral membrane proteins (Cleves et al., 1989; Whitters et al., 1993).

PIPs are phosphorylated derivatives of phosphatidylinositol (PtdIns) that serve a variety of primary intracellular roles in the specification of dedicated membrane microdomains that organize signal transduction processes, as co-factors for the regulated activities of proteins, and as precursors for second messengers such as diacylglycerol and soluble inositol phosphates (Fruman et al., 1998; Di Paolo and De Camilli, 2006). In plant cells, all PI except PI (3, 4 and 5) P3 have been identified. Several lines of evidence suggest that as in yeast, PIs in plants may regulate many cellular activities such as vesicle trafficking (Matsuoka et al., 1995; Kim et al., 2001), pollen tube growth (Kost et al., 1999), and responses to stress and hormonal treatments (Meijer et al., 1999, 2001; DeWald et al., 2001). A number of kinases and phospholipase Cs involved in the metabolism of PIs have been characterized in plants (Cleves et al., 1991; Stevenson et al., 2000).

Homologs of the Sac1 PIP phosphatase are disseminated throughout the eukaryotic kingdom as evidenced by inspection of insect, plant, and mammalian genomes (Figure 1). The intracellular distribution of Sac1 PIP phosphatases in the endoplasmic reticulum (ER) and golgi of mammalian cells recapitulates aspects of what is observed in yeast (Xie et al., 1998; Nemoto et al., 2000), but with some unanticipated twists. Of interest, hSac1 also contains a C-terminal KXKXX motif that serves as binding site for the coatomer (COPI) complex. This COPI interaction motif is a unique feature of mammalian Sac1s as it is not conserved in yeast, plant or Drosophila Sac1 proteins (Figure 1).

Our examinations reveal that *BrSAC1* plays a role in salt, cold and drought responses, as each of these processes regulated *BrSAC1* expression. Rapid induction of *BrSAC1* protein was seen within 1 h of both salt (NaCl) and cold (4°C) treatments. Although *BrSAC1* is rapidly induced by both of these stresses, the pattern of transcript induction over 24 h between them is quite different. *BrSAC1* induction by cold was rapid and peaked at 17-fold in the first 2 h, then declined rapidly until it was close to pre-induced levels by 24 h (Figure 3). The difference in transcript induction pattern may suggest different modes of action for *BrSAC1* in response to stress. Stress responses, such as salt and cold, have been proposed to be activated through different signaling pathways: ABA dependent or ABA independent (Xiong et al., 2002; Shinozaki et al., 2003; Bartels and Sunkar, 2005; Tuteja, 2007). The influence of *BrSAC1* on responses to abiotic stresses of salt, cold and dehydration were further examined through the creation of several overexpressing transgenic lines in tobacco.

In conclusion, we cloned, characterized, and investigated the function of Sac1; PIP phosphatase encoded by the *BrSAC1* gene of *B. rapa* L. Overexpression of the *BrSAC1* gene in tobacco plants enhanced their tolerance to salt, cold and dehydration stresses. These results suggest that *BrSAC1* may be important to the understanding, and potential breeding, of plants towards improved abiotic stress response.

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