J. Plant Biol. (2009) 52:141–146
DOI 10.1007/s12374-009-9015-0

ORIGINAL RESEARCH

Identification of a Novel Transcription Factor, *AtBSD1*, Containing a BSD Domain in *Arabidopsis thaliana*

Jaekyun Park · Mi Jung Kim · Su Jin Jung · Mi Chung Suh

Received: 5 January 2009 / Revised: 2 February 2009 / Accepted: 4 February 2009 / Published online: 4 March 2009

Abstract Transcription factors (TFs) are the key regulators of gene expression and play crucial roles during plant growth and development. A novel TF family, containing one or two BSD domains in a variety of organisms ranging from prokaryotes to human, was newly identified by computational analysis. In this study, one *Arabidopsis* gene encoding the BSD domain, designated as *AtBSD1*, was characterized. The *AtBSD1* transcript was expressed in all *Arabidopsis* tissues tested. The expression level of the *AtBSD1* transcripts was not controlled by exogenous application of abiotic stresses and plant hormones. When the AtBSD1::GFP construct under the control of the CaMV35S promoter was introduced into onion epidermal cells, a GFP signal was detected in the nucleus. A transcriptional activity assay of the AtBSD1 protein in yeast revealed that the AtBSD1 protein functions as a transcriptional activator, and the N-terminal region (1–204 amino acids) of the AtBSD1 protein contains a transcriptional activation domain. These results suggest that *Arabidopsis* genes encoding the BSD1 domain might be classified as a novel transcription factor family.

Keywords *Arabidopsis thaliana* · BSD domain · Transcription · Transcription factor

J. Park · S. J. Jung · M. C. Suh (✉)
Department of Plant Biotechnology and Agricultural Plant Stress Research Center, College of Agriculture and Life Sciences, Chonnam National University,
Gwangju 500-757, Republic of Korea
e-mail: mcsuh@chonnam.ac.kr

M. J. Kim
School of Life Sciences and Biotechnology, Korea University, Seoul 136-701, Republic of Korea

Transcription factors (TFs) are proteins that are involved in the regulation of gene expression, bind to the promoter regions of genes, and control the transcription of downstream genes. TFs consist of two functional regions, a DNA-binding region and an activator region. It was recently reported that the *Arabidopsis* genome encodes approximately 2,000 TFs, which are classified into 68 gene families, mainly according to the structure of the DNA-binding region (Riano-Pachon et al. 2007). About 45% of *Arabidopsis* TFs are plant-specific, whereas the rest of them share DNA-binding domains common to other eukaryotes (Riechmann et al. 2000; Riechmann and Ratcliffe 2000; Riechmann 2002; Gong et al. 2004).

A novel TF family containing the BSD domain, which is not included among the *Arabidopsis* 68 TF gene families, has recently been grouped by BLAST and Hidden Markov Model (HMMer) searches (Doerks et al. 2002; Eddy 1998). The BSD domain is characterized by three α helices that are probably involved in DNA binding and by conserved tryptophan and phenylalanine residues located at the C-terminus of the domain. TFs harboring the BSD domain are known to be present in a variety of species ranging from primal protozoans to humans. The newly discovered BSD domain was named from mammalian *B*TF2 transcription factor, *Drosophila s*ynapse-associated proteins, and *D*OS2-like proteins (Doerks et al. 2002; Fischer et al. 1992). *Arabidopsis* harbors 11 genes encoding the BSD domain, but their functional roles and domains have not been investigated.

In this study, we found that the *AtBSD1* containing the BSD domain functions as a transcriptional factor. The AtBSD1 protein was predominantly localized in the nucleus. By using a β-galactosidase assay in yeast, we demonstrated that the N-terminal region (1–203 AA) of the AtBSD1 protein contains a transcriptional activation domain. Potential

roles of AtBSD1 in transcriptional regulation in *Arabidopsis* are discussed.

Materials and Methods

Plant Materials

Arabidopsis thaliana (ecotype Columbia-0) plants were grown in the culture room under a photocycle of 16 h of light (24°C) and 8 h of darkness (22°C). The *atbsd1* mutant seeds were germinated on the 1/2 MS media with a supplement of 50 μg/ml kanamycin. For exogenous application of abiotic stresses and plant hormones, *Arabidopsis* seeds were germinated on 1/2 MS media for 3 days and then transferred to 1/2 MS liquid media supplemented with 1 μM indole-3-acetic acid, 1 μM gibberellic acid, 1 μM jasmonic acid, 1 μM zeatin, 1 μM abscisic acid, 200 mM NaCl, 200 mM mannitol, or 20% PEG, and incubated for 6 h.

RNA Extraction and RT-PCR

Total RNAs were isolated using TRIzol reagent (Invitrogen, USA) according to the instructions of the manufacturer. Reverse transcription was performed as described in the protocol of the manufacturer (Invitrogen, USA). Primers used in RT-PCR are shown in Table 1. The PCR reaction was conducted in a final volume of 20 μl containing 100 ng of cDNA, 1× *i* Taq buffer (iNtRON, Republic of Korea), 2.5 mM of each dNTP, 1 unit of *i*-Taq polymerase (iNtRON, Republic of Korea), and 10 pmol of each primer.

Isolation of the AtBSD1 cDNA and Subcellular Localization Assay

The coding region of the *AtBSD1* cDNA was amplified using the *AtBSD1*-gene specific primers, At1g10720FF and At1g10720FR, and cloned into pGEMT-easy vector (Promega, USA). The entire nucleotide sequence was confirmed by sequencing. The cloned *AtBSD1* cDNA was digested with *Bam*HI and subcloned into the *Bam*HI-digested pBIN35S-mGFP4 vector (Davis and Vierstra 1998). The resultant plasmid was linearized with *Eco*RI. Particle bombardment was performed as described (Kim et al. 2007) using a biolistic helium gun device (Bio-Rad PDS-1000/He). After bombardment, samples were incubated for 24 h at 25°C in the dark and were observed under a confocal laser-scanning microscope (Olympus BX51, Japan).

β-Galactosidase Assay in Yeast

To construct recombinant plasmids (pAtBSD1-F, pAtBSD1-N, and pAtBSD1-C), the *AtBSD1* cDNA in pGEMT-easy vector (Promega, USA) was amplified using the following primer sets: the At1g10720FF and At1g10720FR2 primers for pAtBSD1-F, the At1g10720FF and At1g10720YR primers for pAtBSD1-N, and the At1g10720YF and At1g10720FR2 primers for pAtBSD1-C. Each PCR fragment was cloned into pGEMT-easy vector (Promega, USA) and was subsequently sequenced. The resultant vectors were digested with *Bam*HI, and approximately 1.3-, 0.6-, and 0.7-kb DNA fragments were eluted and cloned into the *Bam*HI-digested pGBKT7 vector (BD Biosciences Clontech, USA). The resultant

Table 1 Primers used in RT-PCR for analysis of expression patterns

Reactions	Primer names	Primer sequences (5′–3′)	[1]Tm (°C)	Enzyme sites
RT-PCR	Actin2F1	CAT CCA AGC TGT TCT CTC CTT GTA C	60	
	Actin2R1	CAG ACA CTG TAC TTC CTT TCA GGT G	60	
	Actin7F1	ATG GCC GAT GGT GAG GAT ATT CAG	60	
	Actin7R1	CGT ACT CAC TCT TTG AAA TCC AC	60	
	At1g10720F	CCT CTT GAT CCT GAC GAA GAT CTT G	60	
	At1g10720R	GCA GAA TCT TCT TCT TCT GGC CAA TC	60	
	rd29AF1	GAT AAC GTT GGA GGA AGA GTC GGC	60	
	rd29AF1	CAG CTC AGC TCC TGA TTC ACT ACC	60	
Subcellular localization	At1g10720FF	CGG GAT CCC AAT CAT AAT GTT TTC GA	62	*Bam*HI
	At1g10720FR	CGG GAT CCA TGT TCC TTT TTG GTC TG	62	*Bam*HI
Yeast one hybrid	At1g10720FR2	CGG GAT CCT TTC ATG TTC CTT TTT GG	62	*Bam*HI
	At1g10720YR	CGG GAT CCC AAG ATC TTC GTC AGG	62	*Bam*HI
	At1g10720YF	TCG GGA TCC GAT TTG GAA ATG AGT G	62	*Bam*HI
Mutant isolation	LBa1	TGG TTC ACG TAG TGG GCC ATC G	62	
Over-expression	CaMV35SF1	GCC TCT GCC GAC AGT GGT CCC AAA G	60	

[1] Tm indicates the annealing temperature used in PCR. Restriction enzyme sites were underlined.

constructs, pAtBSD1-F, pAtBSD1-N, and pAtBSD1-C, were transformed into yeast strain Y190 (*MATa*, *HIS3*, *lacZ*, *trp1*, *leu2*, *cyhr2*) according to the instructions of the manufacturer (BD Biosciences Clontech, USA). Transformants were selected on selective medium (SD-Trp) supplemented with 25 mM 3-amino-1, 2, 4-aminotriazole (3-AT). For β-galactosidase assays, transformants were cultured on selective medium (SD-Trp-His) for 1 day at 30°C, and filter-lift assays for blue color development were performed for 4 h at 37°C, as described by Breeden and Nasmyth (1985).

Isolation of the *atbsd1* Mutant and Development of Transgenic Lines Overexpressing AtBSD1

T-DNA tagged *atbsd1 Arabidopsis* mutant seeds (SALK_069095) were obtained from the Arabidopsis Biological Resource Center (http://www.arabidopsis.org). Kanamycin-resistant seedlings were screened in order to isolate homozygote lines by PCR screening using the left border primer (LBa1) and the *atbsd1* gene-specific primers, At1g10720F and At1g10720R.

For the development of transgenic lines overexpressing *AtBSD1*, pAtBSD1-F was digested with *Bam*HI. Approximately 1.3-kb DNA fragments were eluted and ligated into *Bam*HI-digested pCAMBIA1300.1 vector. The constructed binary vectors were transformed into *Agrobacterium* strain GV3101 using the freeze–thaw method (An 1987). *Arabidopsis* wild type was then transformed according to the vacuum infiltration method, as described by Clough and Bent (1998). Seeds that had been bulk-harvested from each pot were sterilized and were then germinated on MS agar medium supplemented with 50 μg/ml kanamycin or 30 μg/ml hygromycin. Surviving T_1 seedlings were transferred to soil, and T_1 plants were used for genetic and phenotypic analyses. The identification of the introduced gene was carried out by PCR using CaMV35SF1 and At1g10720R primers.

Results and Discussion

Isolation and Characterization of the *AtBSD1* cDNA

BSD domain genes have been reported to exist in the *Arabidopsis* genome (Doerks et al. 2002). However, no reports have systematically analyzed the BSD domain genes in *Arabidopsis*. We performed BLAST searches to find the BSD domain genes and found that there were 11 BSD domain genes present.

We selected an *Arabidopsis* gene (At1g10720), designated as *AtBSD1*, containing a BSD domain in order to characterize the function of the BSD domain genes. The *AtBSD1* cDNA was isolated from *Arabidopsis* seedlings, where the *AtBSD1* gene was expressed, according to the information in the *Arabidopsis* microarray analysis database (http://www.arabidopsis.org). As shown in Fig. 1a, the AtBSD1 protein (429 amino acids) contains acidic amino-acid-rich sequences, a characteristic feature of a transcriptional activation domain and one BSD domain in its center (Fig. 1a). No putative nuclear localization signal sequence was identified.

Homology searches revealed that the AtBSD1 was matched to proteins with a BSD domain from various plant species (*Vitis vinifera*, *Oryza sativa*, *Picea sitchensis*, *Medicago truncatula*, and *Populus trichocarpa*), moss (*Physcomitrella patens*), amoeba (*Dictyostelium discoideum*), and green alga (*Chlamydomonas reinhardt*), suggesting that AtBSD1 is evolutionarily conserved (Fig. 1b).

Expression of the *AtBSD1* Gene

To investigate the expression of *AtBSD1* gene, RT-PCR was performed. The AtBSD1 transcripts were constitutively expressed in all *Arabidopsis* tissues tested (Fig. 2a). Then, we examined the effects of stresses and hormones on the expression of AtBSD1 gene. The results showed that the *AtBSD1* expression was not regulated by salt and drought stresses in the *rd29A* gene-induced condition as well as various plant hormones (Fig. 2b, c). This finding suggests that it may be a housekeeping gene. This observation is consistent with the *Arabidopsis* microarray analysis from various tissues and 10-day-old seedlings treated with an exogenous application of abiotic and biotic stresses (http://www.bar.utoronto.ca/efp/cgi-bin/efpWeb.cgi).

Subcellular Localization of the AtBSD1 Protein

To investigate the subcellular localization of the AtBSD1 protein, AtBSD1::mGFP was transiently expressed in onion epidermal cells. After incubation in the dark for 24 h, the onion cells were visualized under a confocal laser scanning microscope. As shown in Fig. 3, the AtBSD1::mGFP fusion protein was predominantly localized to the nuclei, whereas mGFP was found throughout the cell. In plants, the typical nuclear localization signal (NLS) was known to be a Pro-Lys-Lys-Lys-Arg-Lys (PKKKRK) (Jans 1995). However, the NLS was not present in the AtBSD1, suggesting that unknown sequences might be involved in the targeting of the AtBSD1 to the nucleus.

AtBSD1 Functions as a Transcriptional Activator, and the N-terminal Region of the AtBSD1 Protein Contains the Transcriptional Activation Domain

Since some BSD domain proteins function as a basal transcription factor and AtBSD1 was localized in the nucleus, we speculated that AtBSD1 may be a transcription

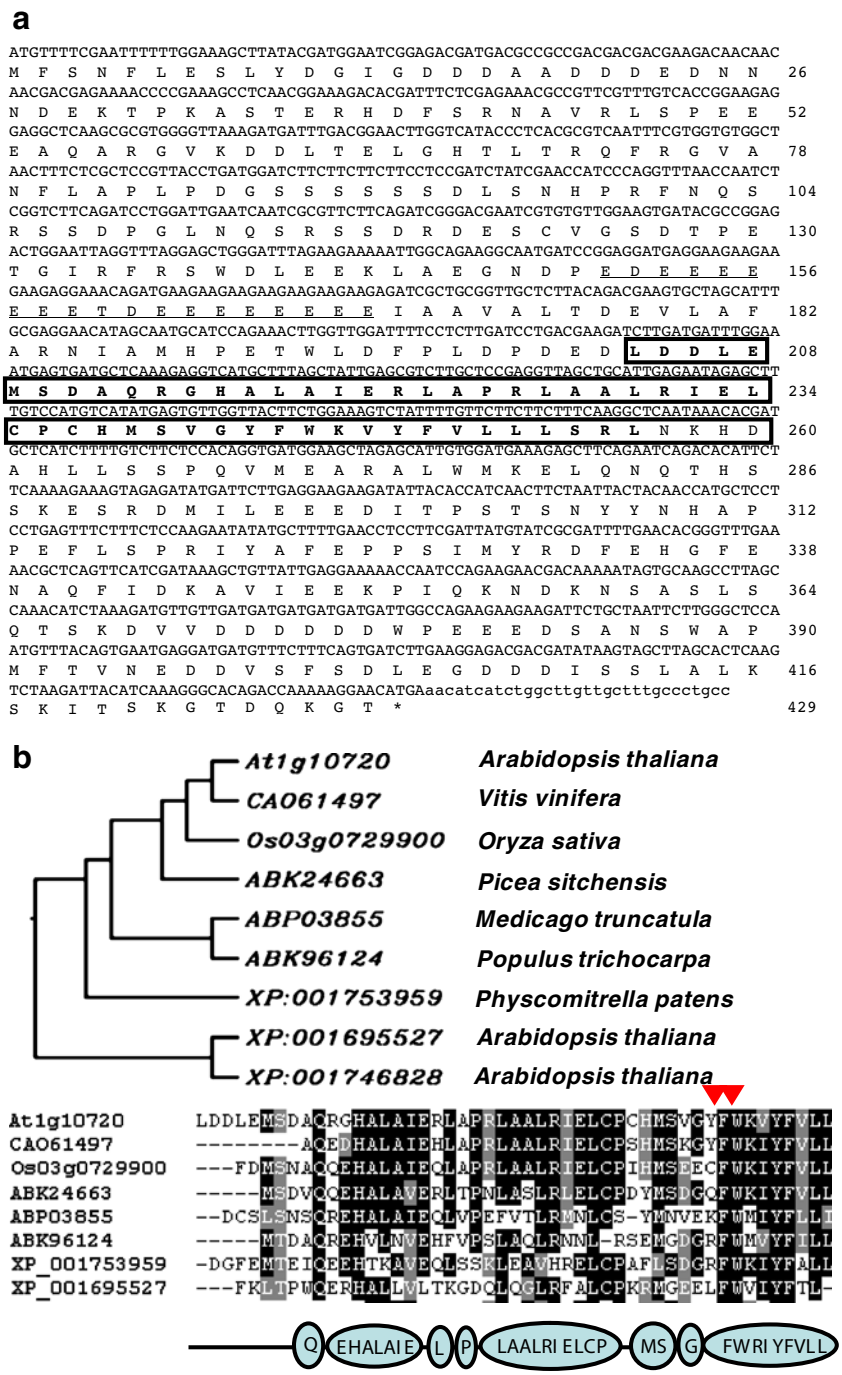

Fig. 1 *AtBSD1* gene. **a** Nucleotide and deduced amino acid (*AA*) sequences of the *AtBSD1* gene. The *numbering on the right* refers to the AA sequences. The putative BSD domain is *boxed*. The location of the acidic AA sequences is *underlined*. **b** Phylogenetic tree of AtBSD1 homologues from various plant species, moss, amoeba, and green alga and alignment of the deduced amino acid sequences of their BSD domains. The conserved amino acid residues in the BSD motif are shown in the *shaded region*. The conserved phenylalanine (*F*) and tryptophan (*W*) residues are indicated by *inverted triangles*

factor that has a transcriptional activity. To determine whether AtBSD1 protein acts as a transcriptional activator and to investigate where its activation domain is if it acts as a transcriptional activator, the full-length (1–429 amino acids) gene and the N-terminal (1–204 amino acids) and C-terminal (205–429 amino acids) regions of the AtBSD1 were subcloned into pGBKT7 plasmid (Fig. 4a). Three resultant plasmids were transformed into yeast. Growth and filter-lift assays showed that full-length and N-terminal region of AtBSD1 had a transcriptional activity (Fig. 4b). In the N-terminal half of AtBSD1, there are acidic amino-acid-rich sequences (amino acids 151–169). It is known that these sequences are among the transcriptional activation domains, which include glutamine- and proline-rich domains depending on the type of amino acid enrichment in the domain (Triezenberg 1995). This result revealed that AtBSD1 functions as a transcriptional activator and that the N-terminal region of AtBSD1 protein contains the transcriptional activation domain.

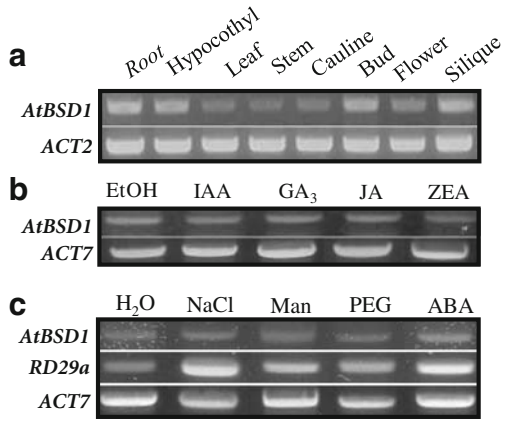

Fig. 2 Expression of the *AtBSD1* gene. **a** Total RNAs were isolated from various *Arabidopsis* tissues, converted into cDNA, and amplified by RT-PCR using gene-specific primers. The *Arabidopsis* actin2 (At3g18780) gene was used as an internal control. **b**, **c** RT-PCR analysis of *AtBSD1* gene expression by exogenous application of various plant hormones and salt and drought stresses (see "Materials and Methods"). The *Arabidopsis* actin7 (At3g18780) gene was used as an internal control. The *rd29A* gene (At5g09810), which is known as a drought stress-inducible gene, was used as a control in the condition of abiotic stresses

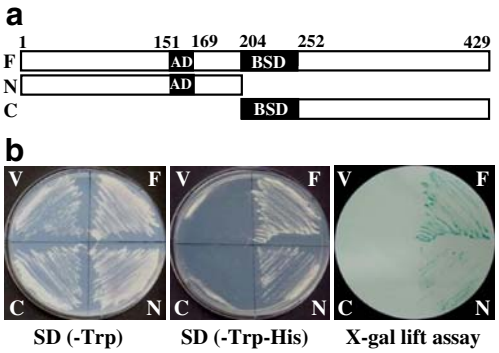

Fig. 4 Transcriptional activation assay of AtBSD1 protein in yeast. **a** Schematic diagram of full-length and deletion constructs of the AtBSD1 in the pGBKT7 vector. **b** Growth of yeast cells in medium SD/-Trp (*left*) and SD/-Trp-His (*middle*) containing 25 mM 3-AT. The X-gal lift assay was carried out using SD/-Trp-His plate (*right*). *V* Yeast transformed with the pGBKT7 vector; *F* yeast transformed with the full-length of the AtBSD1 in the pGBKT7 vector; *C* yeast transformed with the C-terminal region of the AtBSD1 in the pGBKT7 vector; *N* yeast transformed with the N-terminal region of the AtBSD1 in the pGBKT7 vector

The BSD domain is present in diverse organisms, including primal protozoans, plants, yeasts, and humans. This suggests a conserved function for the domain (Doerks et al. 2002). BTF2 is a transcription factor that is essential for the initiation of transcription by RNA polymerase B (Fischer et al. 1992). Synapse-associated proteins function as important molecular components of the nervous system (Reichmuth et al. 1995). DOS2 is known to be required for silencing of all heterochromain regions in yeast. It has been found that deletion of DOS2 results in defects in chromosomal segregation and telomere clustering (Li et al. 2005). Therefore, the BSD domain is found in proteins with diverse functions. This prompted us to question the function of *AtBSD1*. To determine function of *AtBSD1*, we examined the *atbsd1* knockout mutant and generated transgenic plants overexpressing *AtBSD1* gene. In *atbsd1*, T-DNA is inserted into the third exon of the *AtBSD1* gene (Fig. 5a). RT-PCR analysis showed that *AtBSD1* is not expressed in the *atbsd1* mutant (Fig. 5b). The mutant was indistinguishable from wild-type plants under normal growth conditions (Fig. 5c). In addition, *AtBSD1* was

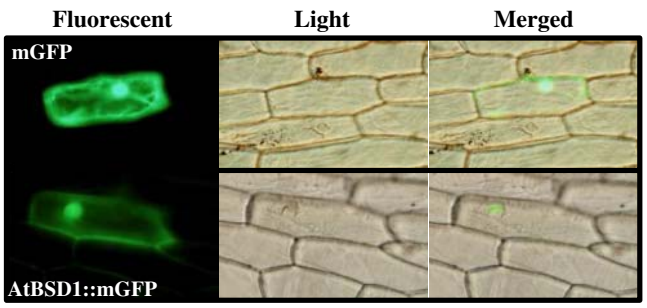

Fig. 3 Subcelluar localization of AtBSD1. The AtBSD1-mGFP fusion construct was introduced into onion epidermis cells by particle bombardment and was visualized under a confocal laser scanning microscope (Olympus BX51, Japan)

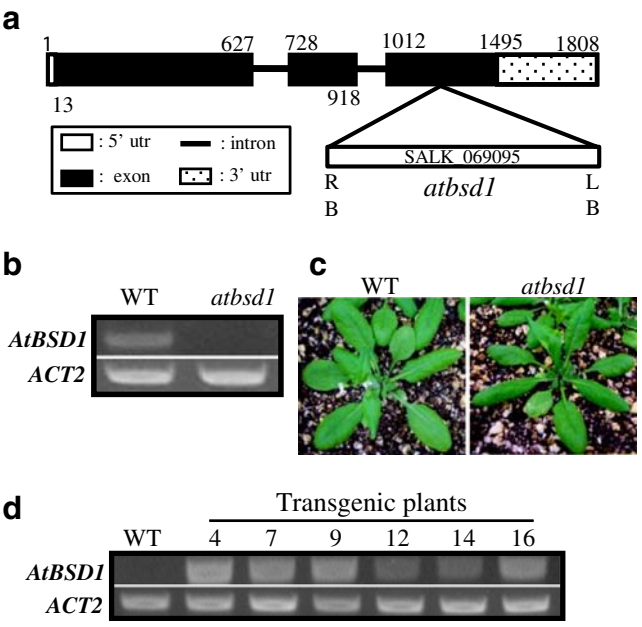

Fig. 5 Genotype and phenotype of the *atbsd1* mutant and transgenic plants overexpressing AtBSD1. **a** The position of the T-DNA insertion site in the *atbsd1* mutant. **b** RT-PCR analysis of the *AtBSD1* gene in the *atbsd1* mutant. **c** Phenotype of the *atbsd1* mutant. The photograph was taken 25 days after sowing. **d** The overexpression of *AtBSD1* in transgenic plants was analyzed by RT-PCR. The *Arabidopsis* actin2 (At3g18780) gene was used as an internal control. *WT* Wild-type plant

overexpressed under the control of 35S promoter. RT-PCR confirmed the overexpression of *AtBSD1* (Fig. 5d). However, the transgenic plants were essentially normal, which suggests that *AtBSD1* may not be the limiting factor for the growth and development of *Arabidopsis* or require other proteins for its function.

Because *AtBSD1* is constantly expressed and has a transcriptional activity, we propose that AtBSD1 may be a basal transcription factor that is involved in the initiation of transcription, as suggested in BTF2 (Fischer et al. 1992). It is also possible that other BSD domain proteins may have redundant functions with AtBSD1. Generation of multiple knockout mutants of BSD domain genes will help to reveal their exact functions.

Acknowledgments We thank the Salk Institute for Genomic Analysis Laboratory for providing sequence-indexed *Arabidopsis* T-DNA insertion mutants of *atbsd1* (SALK_069095). This work was supported by grants from the Agricultural Plant Stress Research Center (R11-2001-09205001-0) of the Korea Science and Engineering Foundation and the Rural Development Administration, Republic of Korea. Acknowledgement is also made to the Bioenergy Research Center, Chonnam National University for its support of this research.

References

An G (1987) Binary Ti vectors for plant transformation and promoter analysis. Methods Enzymol 153:292–305

Breeden L, Nasmyth K (1985) Regulation of the yeast HO gene. Cold Spring Harb Symp Quant Biol 50:643–650

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

Davis SJ, Vierstra RD (1998) Soluble, highly fluorescent variants of green fluorescent protein (GFP) for use in higher plants. Plant Mol Biol 36:521–528

Doerks T, Huber S, Buchner E, Bork P (2002) BSD: a novel domain in transcription factors and synapse-associated proteins. Trends Biochem Sci 27:168–169

Eddy SR (1998) Profile hidden Markov models. Bioinformatics 14:755–763

Fischer L, Gerard M, Chalut C, Lutz Y, Humbert S, Kanna M, Chambon P, Eqly JM (1992) Cloning of the 62-kilodalton component of basic transcription factor BTF2. Science 257:1392–1395

Gong W, Sen YP, Ma LG, Pan Y, Du YL, Wang DH, Yang JY, Hu LD, Liu XF, Dong CX, Ma L, Chen Y-H, Yang X-Y, Gao Y, Zhu D, Tan X, Mu J-Y, Zhang D-B, Liu Y-L, Dinesh-Kumar SP, Li Y, Wang X-P, Gu H-Y, Qu L-J, Bai S-N, Lu Y-T, Li J-Y, Zhao J-D, Zuo J, Huang H, Deng XW, Zhu YX (2004) Genome-wide ORFeome cloning and analysis of Arabidopsis transcription factor genes. Plant Physiol 135:773–782

Jans DA (1995) The regulation of protein transport to the nucleus by phosphorylation. Biochem J 311:705–716

Kim MJ, Kim JK, Shin JS, Suh MC (2007) The SebHLH transcription factor mediates trans-activation of the SeFAD2 gene promoter throught binding to E- and G-box elements. Plant Mol Biol 64:453–466

Li F, Goto DB, Zaratiegui M, Tang X, Martienssen R, Cande WZ (2005) Two novel proteins, Dos1 and Dos2, interact with Rik1 to regulate heterochromatic RNA interference and histone modification. Curr Biol 15:1448–1457

Riano-Pachon DM, Ruzicic S, Dreyer I, Mueller-Roeber B (2007) PlnTFDB: an integrative plant transcription factor database. BMC Bioinformatics 8:42–51

Riechmann JL (2002) Transcriptional regulation: a genomic overview. In: Somerville CR, Meyerowitz EM (eds) The *Arabidopsis* book. American Society of Plant Biologists, Rockville, MD, pp 1–46

Riechmann JL, Ratcliffe OJ (2000) A genomic perspective on plant transcription factors. Curr Opin Plant Biol 3:423–434

Riechmann JL, Heard J, Martin G, Reuber L, Jiang CZ, Keddie J, Adam L, Pineda O, Ratcliffe OJ, Samaha RR, Creelman R, Pilgrim M, Broun P, Zhang JZ, Ghandehari D, Sherman BK, Yu G-L (2000) *Arabidopsis* transcription factors: genome-wide comparative analysis among eukaryotes. Science 290:2105–110

Reichmuth C, Becker S, Benz M, Debel K, Reisch D, Heimbeck G, Hofbauer A, Klagges B, Pfluqfelder GO, Buchner E (1995) The sap47 gene of Drosophila melanogaster codes for a novel conserved neuronal protein associated with synaptic terminals. Brain Res Mol Brain Res 32:45–54

Triezenberg SJ (1995) Structure and function of transcriptional activation domains. Curr Opin Genet Dev 5:190–196