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Research Article

A transcription factor "*OsNAC075*" is essential for salt resistance in rice (*Oryza sativa* L.)

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Abstract Salt stress is a major environmental factor influencing plant growth and development. To identify salt tolerance determinants, we systematically screened *salt sensitive* rice mutants by use of the Activator/Dissociation (*Ac/Ds*) transposon tagging system. In this study, we focused on the salt sensitive mutant line, designated SSM-1. A gene encoding a NAC transcription factor homologue was disrupted by the insertion of a *Ds* transposon into SSM-1 line. The *OsNAC075* gene (EU541472) has 7 exons and encodes a protein (486-aa) containing the NAC domain in its N-terminal region. Sequence comparison showed that the *OsNAC075* protein had a strikingly conserved region at the N-terminus, which is considered as the characteristic of the NAC protein family. *OsNAC075* protein was orthologous to *Arabidopsis thaliana ANAC075*. Phylogenetic analysis confirmed *OsNAC075* belonged to the *OsNAC3* subfamily, which plays an important role in response to stress stimuli. RT-PCR analysis showed that the expression of *OsNAC075* gene was rapidly and strongly induced by stresses such as NaCl, ABA and low temperature (4℃). Our data suggest that *OsNAC075* holds promising utility in improving salt tolerance in rice.

Keywords *Ds* insertion mutant, *OsNAC075*, rice, salt stress, transcription factor

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Introduction

Numerous biotic and abiotic stress factors such as drought, cold, high salinity, insect feeding and pathogen infection constantly influence plant growth and development, and are major limit factors on crop productivity. Many genes that are induced by various abiotic stresses have been identified using microarray analysis techniques (Fowler and Thomashow 2002; Seki et al. 2002b; Rabbani et al. 2003; Bray 2004; Yamaguchi-Shinozaki and Shinozaki 2006). That genes are thought to function in stress tolerance as a regulation of gene expression and signal transduction in the response to stress (Xiong et al. 2002; Shinozaki et al. 2003). Gene expression and regulation is mediated essentially by the change in the level and/ or activity of gene-specific transcription factors. The NAC gene family is one of the transcription factors and widely distributed in plant species. These genes contain a highly conserved at their N-terminus, referred to NAC domain (for *NAM*, *ATAF*1/2, *CUC*2) and a highly diverged C-terminus as transcriptional activation region (Ren et al. 2000; Xie et al. 2000; Duval et al. 2002). Up to date, the members of NAC gene family have been shown to be involved in many processes of plant development and environmental stresses; lateral root formation in auxin signaling (Xie et al. 2000), leaf senescence (John et al. 1997), formation of flower organ primordial (Sablowski and Meyerowitz 1998), and responses to biotic or abiotic stresses (Hegedus et al. 2003; Fujita et al. 2004), maintenance of the shoot apical meristem (SAM) (Souer et al. 1996; Aida et al. 1997; Weir et al. 2004), formation of flower organ primordia (Sablowski and Meyerowitz 1998), hormone signaling (Xie et al. 2000; Che et al. 2002; Greve et al. 2003), response to pathogen infection (Ren et al. 2000; Collinge and Boller 2001; Selth et al. 2005), leaf senescence (John et al. 1997), embryo development (Duval et al. 2002), transportation

of mRNA over long distances via the phloem (Ruiz-Medrano et al. 1999) and responses to different abiotic stresses (Hegedus et al. 2003; Tran et al. 2004). Rice (*Oryza sativa* L.), one of the most important crops, is an ideal model species for the study of crop genes due to its commercial value, relatively small genome size (ca.430 Mb), diploid origin (2x = 24) and close relationship to other important cereal crops. Insertional mutagenesis based on T-DNA and maize Activator/Dissociator (*Ac*/*Ds*) transposons is most suitable for a systematic functional analysis of multifamily plant genes. In the previously studies, we used the Activator/Dissociation (*Ac*/*Ds*) two-component transposon system (Han 2002) in rice to prepare a large collection of gene-trapped *Ds* insertion lines of rice carrying a single *Ds* insertion (Chin et al. 1999; Kim et al. 2002, 2004).

To analyze the functions of nuclear genes, we screened salt sensitive mutants from *Ds*-tagged rice lines. Among 20,000 *Ds*-tagged lines, we selected one line with salt sensitive phenotype closely linked to insertion sites of the *Ds* element, and termed them SSM-1 (for salt sensitive mutant). In this study, we focus on salt sensitive mutant, named SSM-1, which has a disrupted gene for an NAC-like protein. Physiological analysis of SSM-1 mutant revealed that *OsNAC075* is essential roles for salt tolerance in rice.

Materials and methods

Selection of salt sensitive mutant

We made crosses between a transgenic line expressing *Ac* transposase as the female parent and *Ds*-GUS-T-DNA lines as pollen parents (Chin et al. 1999). Selection of the transposed lines was described previously (Han 2002.). *Ds-insertional T4 seeds* were surface-sterilized in a solution of Clorox plus 0.01% Triton X-100 for 10 min, washed with sterilized water three times, planted in rows onto MS (Murashige and Skoog 1962) medium containing 10mM NaCl, 3% Sucrose and 1.2% agar, pH 5.7. The plates were incubated at 22℃ under continuous illumination. Plates were placed in a vertical position to allow roots to grow along the agar surface toward gravity. Putative mutant seedlings were picked up 2 week later and transferred onto a 0.6% agar medium without NaCl. When appropriate, seedlings were transplanted to pots, grown to maturity and harvested to next generation seeds. T5 seeds selected were defined to MS medium containing various NaCl concentrations by the root-bending assay (Wu et al. 1996).

TAIL-PCR and DNA gel blot

TAIL-PCR, and DNA gel blot analysis were carried out as described by Tsugeki et al (1996), cloned into the pGEM-T easy vector system (Promega, USA) and sequenced. The sequences were analyzed online at the following website: http://www.ncbi.nlm.nih.gov, http://www.gramene.org.

Gene cloning

The first-strand cDNA was generated by using Superscript^{TM} II Reverse Transcriptase according to the manufacturer's instructions (TaKaRa, Japan). Two degenerate oligonucleotide primers Fw1 (5'-ATAAGATTGGCCA AGTTGATGTC-3') and Rv1 (5'-TTCTTGGTGAAAT GCCATAC-3') were designed and synthesized according to the of flanking sequences revealed the gene locus of AP002743 on chromosome 1, which was annotated by the RGAD Rice Genome Annotation Database (Sasaki et al. 2002, http://rgp.dna.affrc.go.jp/RiceHMM/). RT-PCR (TaKaRa, Japan) was used to amplify the core conserved fragment from total RNA of 'Japonica cultivar's Dongjin,' leaves by denaturing the template at 94℃ for 3 min and then followed by 30 cycles of amplification (94℃ for 45 s, 56℃ for 45 s and 72℃ for 1 min) and by 8 min at 72℃ after reverse transcription of the total RNA at 50℃ for 40 min. Sequence comparison of this cDNA fragment with other plant NAC gene sequences indicated that this fragment already included the 3′ end of the NAC-like gene. Rapid amplification of cDNA ends (RACE) was performed to amplify its unknown 5′ end. The first strand cDNA (5′-ready cDNA) synthesis in 5′ RACE was performed according to the manual of the SMART^{TM} RACE cDNA Amplification Kit (Clontech Laboratories INC., USA) using the 5′-RACE CDS Primer (provided by the Kit). Based on the flanking sequence of *OsNAC075* gene, the complementary reverse gene specific primer Rv2 (5′- GAGGCTGCGTCTGGT AGAAG-3′) was designed and synthesized. The 5′ RACE-PCR was carried out with primers Rv2 and the forward primer UPM through using the 5′-ready cDNA as the template by denaturing the cDNA at 94℃ for 3 min followed by 35 cycles of amplification (94℃ for 45 s, 52℃ for 45 s, 72℃ for 90 s) and by 8 min at 72℃. Then the nested-PCR was performed with the NUP as the forward primer and n-Rv2 (5′- TCACCCAGTTG GTCTTCTCC-3′) as the reverse primer by denaturing

the template at 94℃ for 3 min followed by 35 cycles of amplification (94℃ for 40 s, 60℃ for 40 s, 72℃ for 90 s) and by 8 min at 72℃. By aligning and assembling the sequences of the RT-PCR and 5′ RACE products, the full-length cDNA of *OsNAC075* was amplified by a simple PCR with the 5′-ready cDNA as the template using specific primers Fv3 (5′-ATGTACACCTC TCCCACACT-3′) and Rv3 (5′-CTATCCATGATGAT CCTGGTTG -3′). The protocol consisted of 35 cycles of amplification (94℃ for 45 s, 60℃ for 45 s, 72℃ for 150 s) followed by 8 min at 72℃. The amplification and sequencing of the full-length cDNA of *OsNAC075* was repeated twice.

Phylogenetic analysis

For phylogenetic analysis, other plant NAC-like gene sequences were retrieved from GenBank database: *Arabidopsis thaliana ANAC* (AY117224), *ATAF1* (X 74755), *ATAF2* (X74756), *AtNAC3* (AB049070), *NAC2* (AF201456), *NAC1* (AF198054), and *ANAC055* (At3g 15500); *Oryza sativa OsNAC*1 (AB028180), *OsNAC2* (AB028181), *OsNAC5* (AB028184), *OsNAC6* (AB028 185), and *OsNAC8* (AB028187); *Triticum sativa GRAB2* (AJ010830); Petunia × hybrid *NAM* (X92205); *Lycopersicon esculentum SENU5* (Z75524); and *Nicotiana tabacum TERN* (AB021178). Using DNAMAN program, overall amino acid sequences were aligned, and then the unrooted phylogenetic tree was generated. This tree was setup with the distance matrix using the neighbor-joining method. Poisson correction with the complete deletion of gaps was used to calculate protein distances. Bootstrap values are based on 100 iterations.

Histochemical analysis

SSM-1 mutant plants harboring *Ds*-GUS construct were generated as described above. For ß-glucuronidase assay, materials were stained at 37℃ overnight in 1 mg/mL 5-bromo-4-chloro-3-indolyl-ß-D-glucuronic acid, 5 mM potassium ferricyanide, 5 mM potassium ferrocyanide, 0.03% Triton X-100, and 0.1 M sodium phosphate buffer, pH 7.0.

Subcellular localization analysis

The full open reading frame (ORF) of *OsNAC075* was amplified using the cDNA clone mentioned above as template. The PCR product was ligated into the pGWB5-GFP vector, resulting in an in-frame fusion protein of GFP (green fluorescent protein) gene and the *OsNAC075* ORF. The construct (p35S:GFP-*OsNAC075*) was transformed into onion epidermal cells by particle bombardment using a Biolistic PDS-1000/He gene gun system (BIO-RAD). After 24 h incubation of transformed onion epidermal cells, GFP signal was detected by a comfocal fluorescence microscope.

Stress treatment and RT-PCR analysis

Rice seedlings were grown on MS agar medium under continuous light and 14-day-old seedlings were treated with NaCl, abscisic acid, and low temperature as described previously (Shi et al. 2000). Determination of gene expression in roots and shoots was performed as described previously (Shi et al. 2000). Total RNA was isolated by hot-phenol method. The RNA was treated with DNase I to remove contaminating DNA and 50 ng aliquot of total RNA was used for RT-PCR analysis. It was performed using SuperScript One-Step RT-PCR with Platinum *Taq* kit (Invitrogen, USA) according to the manufacturer's manual with a gene specific primer sets and 10 µl of each reaction mixture was run on 1.2% agarose gel. Optimum cycle numbers of PCR were determined in the preliminary experiment and amplified DNA fragments were sequenced to confirm them as the target genes. To standardize the results, the relative abundance of Actin 2 gene was also determined as the internal standard. The following primers were used for the gene specific primers, i.e. *OsNAC075*: 5′-AGCA CCACCAGACCTACGTGA-3′ and 5′-CAAGGTCCTT GAGCTCGGG-3′, *Actin2*: 5′-CGCCCGTCATCGTGCG GTTC-3′ and 5′-TCCGCGGGGTCCATGGTC

Results

Selection of a salt sensitive mutant

A salt sensitive mutant, SSM-1, isolated from 20,000 seedlings of our *Ds*-tagged lines, showed inhibition of root growth on Murashige and Skoog (1962) (MS) nutrient medium supplemented with different concentration of NaCl (Fig. 1). On MS nutrient medium, the aerial parts of SSM-1 mutant was indistinguishable from those of the wild type, but the roots of the mutant grew more slowly than did wild-type roots. Upon transfer to medium supplemented with 100 mM NaCl, the growth of both

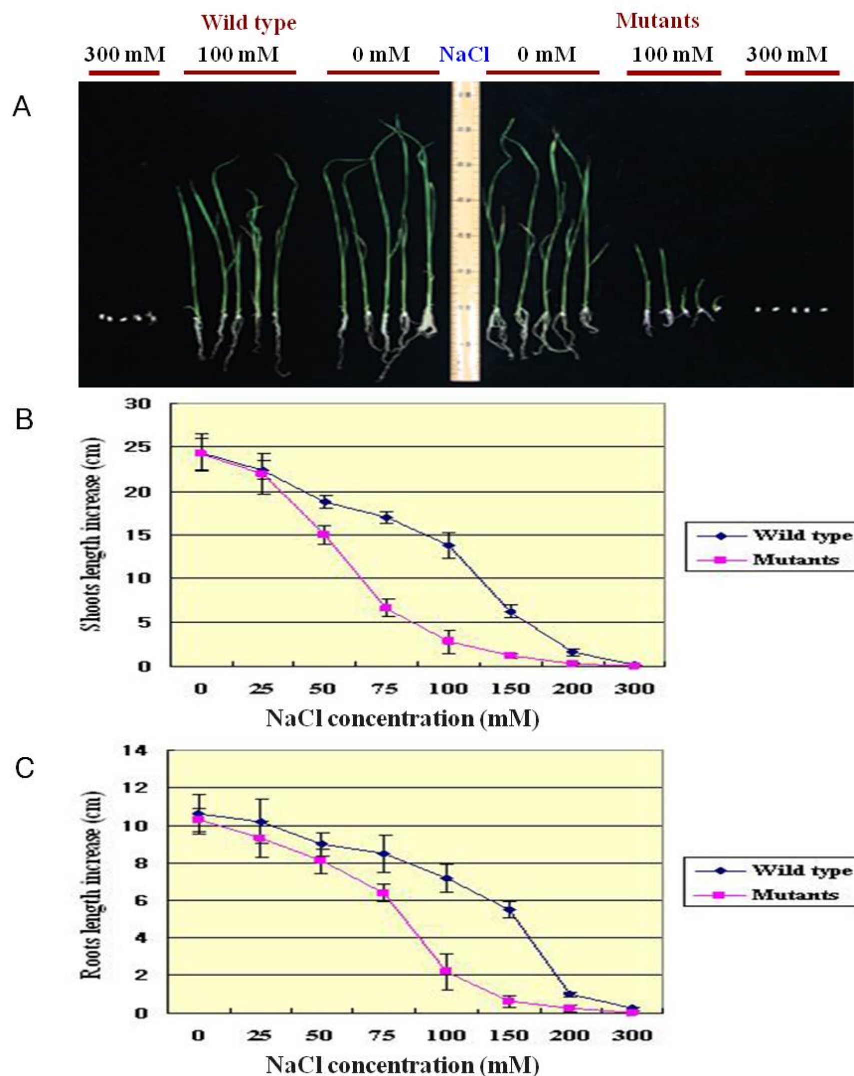

Fig. 1 Effect of NaCl on wild-type and mutant seedlings grown in high and low concentration availability. (A) The phenotype of the *SSM-1* mutant and wild-type under the conditions of NaCl stress. (B, C) Seedlings were grown on agar plates containing different concentrations of NaCl for two weeks

the shoot and the root of mutant plants were inhibited to a greater extent than the growth of those of wild-type plants (Fig. 1B, C).

Identification of NAC gene from SSM-1 mutant

To identify the locus in which the *Ds* transposon inserted, DNA fragments adjacent to the 5' and 3' ends of the inserted *Ds* were amplified from SSM-1 mutant seedlings using the thermal asymmetric interlaced (TAIL) polymerase chain reaction (PCR) technique (Liu and Whittier 1995). Analysis of flanking sequences revealed the gene locus of AP002743 on chromosome 1, which was annotated by the RGAD Rice Genome Annotation Database (Sasaki et al. 2002, http://rgp.dna.affrc.go.jp/RiceHMM/) (Fig. 2A). Based on the sequence of flanking region, we obtained the 1,461-bp full-length cDNA of putative NAC-like gene in rice by RACE, tentatively designated as *OsNAC075* (GenBank accession no. EU541472). The cDNA of *OsNAC075* contains a 1461-bp open reading frame encoding a protein of 486 amino acids with a calculated molecular mass of 54.3 kDa and an isoelectric point of 7.97. In SSM-1 plants, the *Ds* transposon was inserted into the second exon of AP002743. Also, *OsNAC075* expression in SSM-1 plants was examined by RT-PCR analysis. We detected *OsNAC075* mRNA in wild-type plants, but not in SSM-1 mutant plants (Fig. 2C). Therefore these results indicate that disruption of the *OsNAC075* gene causes a salt sensitive phenotype. Also, promoter-glucuronidase (GUS) fusion analysis

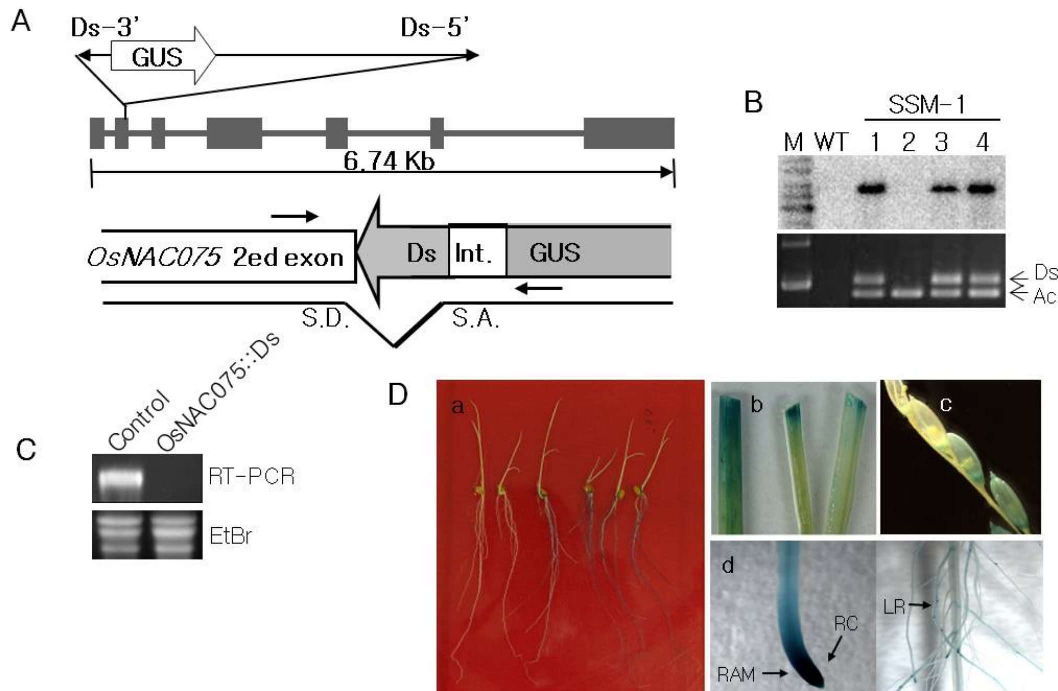

Fig. 2 Transcript analysis of *OsNAC075* gene in SSM-1 line. A, Genomic structure of *OsNAC075* showing the position of the trap *Ds* element. The putative splicing donor (S.D.) and acceptor sites (S.A.) are indicated by the bent arrow. B, Southern hybridization of *Eco*RI-digested DNA from *OsNAC075* plants was performed using probes recognizing a 1.2 kb GUS-encoding region and PCR analysis from *OsNAC075* genomic DNA. C, RT-PCR analysis of *OsNAC075* mRNA. D, Histochemical analysis of T2 (*OsNAC075*::*Ds)* line. (a) Whole plants, (b) Leaf sheath, (c) Stamen and pistil in seed, (d) Shoot and root tip

was performed to investigate the potential developmental and tissue-specific expression in SSM-1 line. GUS staining was detected throughout the SSM-1 plants harboring the *OsNAC075* promoter-*Ds*-GUS fusion construct (Fig. 2D), suggesting that promoter of *OsNAC075* is expressed ubiquitously. SSM-1 mutant line showed root specific GUS staining in the T1 and T2 generations by histochemical analysis of their progeny. Examination of 20 independent SSM-1 mutant lines shown that GUS activity was consistently localized in young adventitious roots, mainly in root apical meristem (RAM), lateral root (LR) tips, longation zones and to lesser extent around junctions of the seminal root (SM) and lateral root. GUS staining was especially intense in emerging lateral and tertiary root's primordia (TRP) (Fig. 2D). Vascular of young root frequently displayed "mosaic" GUS staining in small-dispersed groups of cells. Older root and root hairs typically showed no GUS staining. Strong GUS staining also was shown at the cutting region edge of leaf blades and leaf sheath, but was not shown at the leaf meristem. These expression patterns indicate a ubiquitous function of *OsNAC075* in rice, consistent with the role of transcription factor as an essential cofactor for numerous enzymes in cellular metabolism and as a regulator of ion transport.

Molecular characterization of OsNAC075 protein

An alignment of the predicted amino acid sequence of *OsNAC075* with the cloned NAC-like genes from many organisms was conducted using the DNAMAN program. As shown in Figure 3, the deduced *OsNAC075* protein contained a highly conserved region in its N-terminal sequence that may function as a DNA-binding domain. The N-terminal 158 residues contained five subdomains (A-E) (Fig. 3) according to Ooka et al. (2003). The C-terminus of *OsNAC075* protein, serving as transcription activation domain, showed low sequence similarity to other plant NAC proteins. It was indicated that NAC proteins may perform different functions accompanied by diverse C-terminal transcriptional activation domains. Analysis showed that *OsNAC075* was closely related to *OsNAC3* with 34.4% identity in their carboxy-terminal domains (Fig. 3). The variable carboxy-terminal domain (residues 159-305) of the *OsNAC075* protein was compared to the corresponding regions of the most closely related plant homologue *OsNAC* (AAX85684) and *OsNAC*-

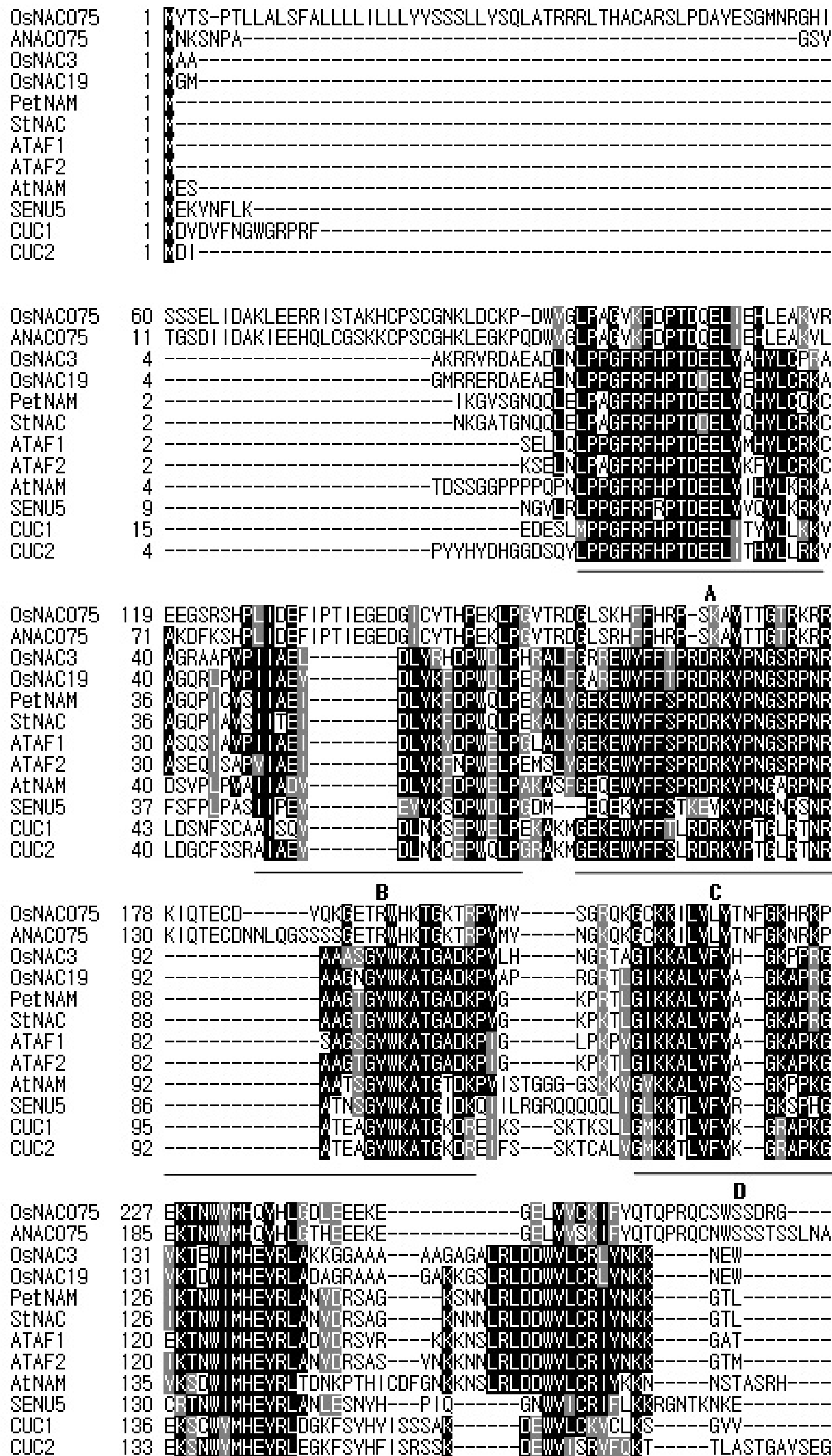

Fig. 3 Sequence alignment of *OsNAC075* protein with other NAC-domain proteins, including *Oriza sativa OsNAC3* (BAA89797), *OsNAC19* (AAT02360); Petunia × hybrida *PetNAM* (AAM34766); *Solanum tuberosum StNAC* (CAC42087); *L. esculentum SENU5* (Z75524); and *A. thaliana ANAC075* (NP_194652), *AtNAM* (AF123311), *ATAF1* (NP_171677), *ATAF2* (NP_680161), *CUC1* (AB049069), and *CUC2* (AB002560). The amino acids with identity over 75% are shaded in black,whereas those with identity between 50 and 75% are shaded in gray. The five subdomains of NAC

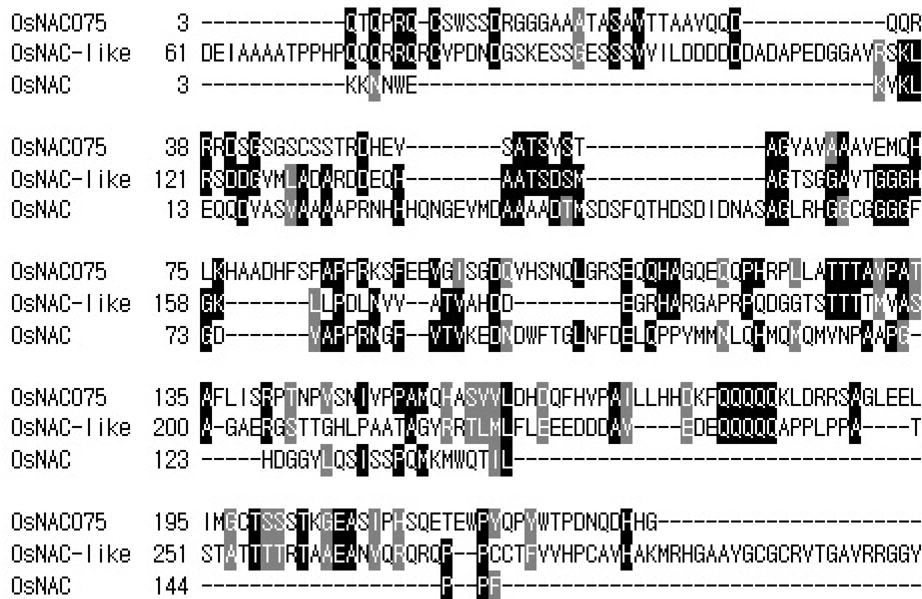

Fig. 4 Alignment of translated *OsNAC075* variable carboxy-terminal domain with corresponding region of the most closely related plant homologue *OsNAC* (AAX85684) and *OsNAC-like* (BAD34064) protein. The identical residues are *highlighted in black*

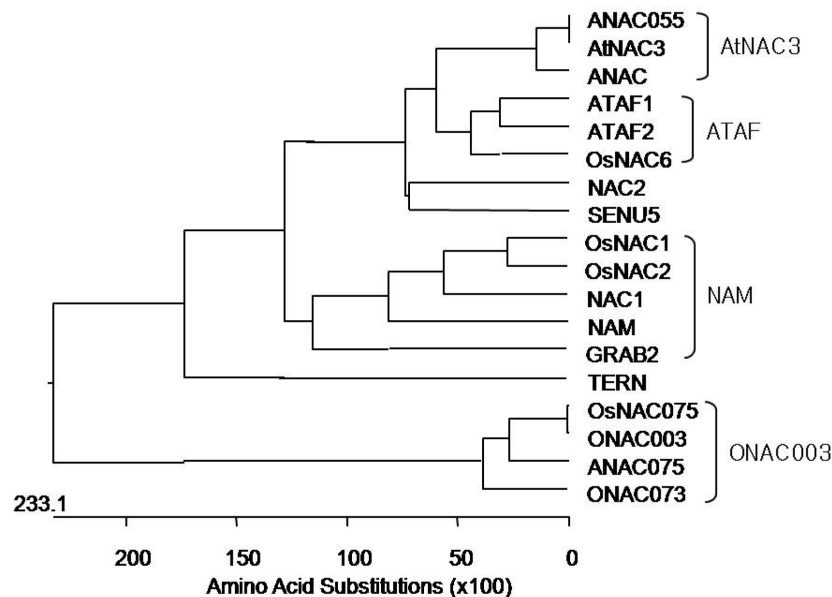

Fig. 5 Phylogenetic relationship between *OsNAC075* protein and other plant NAC-domain proteins

like (BAD34064) protein (Fig. 4). To confirm this relationship, phylogenetic analysis was performed using overall amino acid sequences of *OsNAC075* and other plant NAC-domain proteins, and the result was slightly different from that of Ooka et al. (2003). To compare simplicity and facility, only major subfamilies were shown in Figure 6. It was indicated that the putative *OsNAC075* protein belonged to the *OsNAC3* subfamily, which was composed of *ANAC075, ONAC003* and *ONAC073* proteins.

Also Southern blot analysis was carried out using specific C-terminal region of probes. The use of probes showed single band, suggesting that each member of *OsNAC075* genes existed as a single copy in rice genome (Fig. 6). This result indicates that nucleotide sequences encoding the NAC domain are highly redundant and the *OsNAC075* genes formed a multigene family in rice genome.

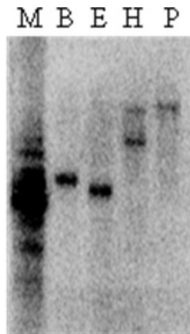

Fig. 6 Southern blot analysis with *OsNAC075* about wild type rice. The specific C-terminal region of *OsNAC075* was used as a probe. Restriction enzymes used were as follows; M: molecular marker, B: *Bam*HI, E: *Eco*RI, H: *Hind*III, P: *Pst* I

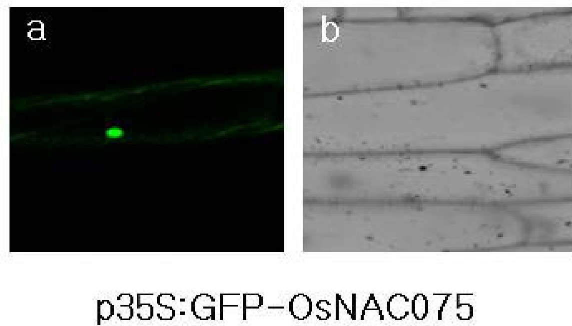

Fig. 7 Subcellular localization of *OsNAC075* gene. p35S:GFP-*OsNAC075* was transiently expressed in onion epidermal cells. The photograph was taken in the dark field for green fluorescence (a), under bright light for the morphology of the cell (b)

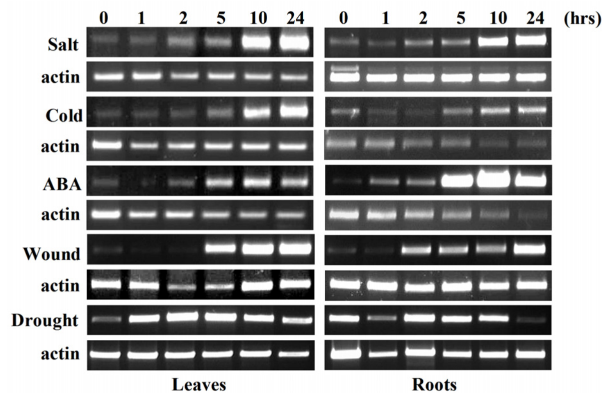

Fig. 8 The semi-quantitative PCR analysis of *OsNAC075* gene expression in response to 250 mM NaCl, 100 mM ABA and temperatures (4℃) at 0, 1, 2, 5, 10, and 24 h after treatments, respectively

Subcellular localization

We analyzed the subcellular localization of *OsNAC075* by particle bombardment transient expression analysis of *OsNAC075*-GFP fusion gene in onion epidermal cells. As shown in Figure 7, cells transformed with *OsNAC075*-GFP displayed fluorescence specifically in the Plasma membrane and nuclear, suggesting that *OsNAC075* is a nuclear-localized protein.

Gene expression under abiotic stresses

To elucidate the expression patterns of *OsNAC075* gene under stresses such as salt (250 mM NaCl), low temperature (4℃), wound, drought and ABA, quantitative RT-PCR analysis was performed. The results showed that *OsNAC075* was up-regulated exclusive of drought stress, and reached the high level at 10 h after treatment. The *OsNAC075* transcripts started to accumulate from 5 h after salt and cold treatment and remained at a high level over the 24-h post treatment period. These results collectively indicated that *OsNAC075* gene expression was preferentially related to salt and cold stress responses and its mediated signaling transduction might be ABA dependent.

Discussion

Plant salt tolerance is a complex trait involving many genes. Even though the entire genome sequence of rice is known, the functional identification of salt tolerance determinants in this model plant remains a formidable challenge. Reverse genetic screens based on plant phenotypes are very powerful because the approach does not depend on previous knowledge and therefore has the potential to reveal unexpected salt tolerance determinants and unsuspected connections. In this report, we describe the isolation salt sensitive rice mutant by screening from ~20,000 *Ds*-tagged rice lines. Also we analyzed this mutant, SSM-1 (Fig. 1), revealing that the mutant is tagged with *Ds* (Fig. 1) at the locus annotated as AP002743 on chromosome 1. A database search and multiple alignment of deduced amino acid sequences of flanking region revealed that this gene (*OsNAC075*) belongs to the family of NAC proteins called with plant-specific transcription factor, which functions in many processes of plant development; lateral root formation in auxin signaling (Xie et al. 2000), leaf senescence (John et al. 1997), formation of flower organ primordial (Sablowski and Meyerowitz 1998), and responses to biotic or abiotic stresses (Hegedus et al. 2003; Fujita et al. 2004), maintenance of the shoot apical meristem

(SAM) (Souer et al. 1996; Aida et al. 1997; Weir et al. 2004), formation of flower organ primordia (Sablowski and Meyerowitz 1998), hormone signaling (Xie et al. 2000; Che et al. 2002; Greve et al. 2003), response to pathogen infection (Ren et al. 2000; Collinge and Boller 2001; Selth et al. 2005), leaf senescence (John et al. 1997), embryo development (Duval et al. 2002), transportation of mRNA over long distances via the phloem (Ruiz-Medrano et al. 1999) and responses to different abiotic stresses (Hegedus et al. 2003; Tran et al. 2004). The amino acid sequence of *OsNAC075* showed high homology with that of *ANAC075*, *ONAC003* and *ONAC073* RF1 from Arabidopsis and rice, and the N-terminal region of *OsNAC075* was shown to function as a DNA-binding domain containing five subdomains: A, B, C, D and E (Fig. 3). It has been reported that subdomain C may be involved in DNA binding (Kikuchi et al. 2000). Subdomain E might be involved in functional and/or developmental stages and/or tissue-specific diversity. It might also be involved in DNA binding in cooperation with subdomain D (Duval et al. 2002). Also the variable carboxy-terminal domain (residues 159-305) of the *OsNAC075* protein was compared to the corresponding region of the most closely related plant homologue *OsNAC* (AAX85684) and *OsNAC-like* (BAD34064) protein. Analysis showed that *OsNAC075* was closely related to *OsNAC3* with 34.4% identity in their carboxy-terminal domains (Fig. 4). Phylogenetic analysis showed that the putative *OsNAC075* protein belonged to the *OsNAC3* subfamily, which plays an important role in response to stress stimuli. It has been reported that members of subgroup *ANAC075*, *ONAC003* and *ONAC073* proteins, could be rapidly and transiently induced by the stress response (Collinge and Boller 2001). Studying salt stress, especially understanding of plant ion homeostasis and osmo-balance, is an important stress that limits plant productivity on agricultural lands. Decades of physiological and molecular studies have generated a large body of literature regarding potential salt tolerance determinants. Recent advances in applying molecular genetic analysis and genomics tools in the model plant *Arabidopsis thaliana* are shading light on the molecular nature of salt tolerance effectors and regulatory pathways. Screening for mutants that are tolerant to high concentrations of salt during germination represented the first such attempt. The germination salt tolerant mutants were named as *rs* (Saleki et al. 1993), *rss* (Werner and Finkelstein 1995), or *san* (Quesada et al. 2000). Other mutant screens used the inhibitory effect of salts on root growth (*sos* mutants) (Zhu 2000) or general seedling growth (Tsugane et al. 1999). The identification of *Salt Overly Sensitive* (*SOS*) loci has uncovered an important regulatory pathway that controls ion homeostasis and salt tolerance (Zhu 2000).

In these studies, we also precisely characterized the phenotype of SSM-1 mutant to analyze the roles of *OsNAC075* in salt stress conditions. Although the SSM-1 mutant could grow on medium supplemented with low NaCl concentrations (Fig. 1B), it showed severe phenotypes: it was seedling-lethal, had small yellow cotyledons and very pale green true leaves. And NaCl and cold induced the expression of *OsNAC075* gene rapidly and strongly. It is indicated that the *OsNAC075* can improve stress tolerance by suppressing the negative effects of *OsNAC075* on growth under normal growth conditions. Collectively, our results indicate that *OsNAC075* functions as a transcriptional activator in response to abiotic stress in plants. We conclude that *OsNAC075* may serve as a useful biotechnological tool for the improvement of stress tolerance in various kinds of plants.

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Literature cited

Aida M, Ishida T, Fukaki H, Fujisawa H, Tasaka M (1997) Genes involved in organ separation in *Arabidopsis:* an analysis of the cup-shaped cotyledon mutant. Plant Cell 9:841-857

Bray EA (2004) Genes commonly regulated by water-deficit stress in *Arabidopsis thaliana*. Journal of Experimental Botany 55:2331-2341

Che P, Gingerich DJ, Lall S, Howell SH (2002) Global and Hormone-Induced Gene Expression Changes during Shoot Development in *Arabidopsis*. Plant Cell 14:2771-2785

Chin HG, Choe MS, Lee SH, Park SH, Koo JC, Kim NY, Lee JJ, Oh BG, Yi GH, Kim SC, Choi HC, Cho MJ, Han CD (1999) Molecular analysis of rice plants harboring an *Ac*/*Ds* transposable element-mediated gene trapping system. Plant J. 19:615-623

Collinge M, Boller T (2001) Differential induction of two potato genes, Stprx2 and StNAC, in response to infection by Phyto phthora infestans and to wounding. Plant Mol

Biol 46:521-529

Duval M, Hsieh TF, Kim SY, Thomas TL (2002) Molecular characterization of AtNAM: a member of the Arabidopsis NAC domain superfamily. Plant Mol Biol 50:237-248

Fowler S, Thomashow MF (2002) *Arabidopsis* transcriptome profiling indicates that multiple regulatory pathways are activated during cold acclimation in addition to the CBF cold response pathway. Plant cell 14:1675-1690

Fujita M, Fujita Y, Maruyama K, Seki M, Hiratsu K, Ohme-Takagi M (2004) A dehydration-induced NAC protein, RD26, is involved in a novel ABA-dependent stress-signaling pathway. Plant J 39:863-876

Greve K, La Cour T, Jensen MK, Poulsen FM, Skriver K (2003) Interactions between plant RING-H2 and plant-specific NAC (NAM/ATF1/2/CUC2) proteins: RING-H2 molecular specificity and cellular localization. Biochem J 371:97-108

Han CD (2002) Rice functional genomics by transposon mutagenesis. Asia Pacific Biotech News 6:930-935

Hegedus D, Yu M, Baldwin D, Gruber M, Sharpe A, Parkin I (2003) Molecular characterization of Brassicanapus NAC domain transcriptional activators induced in response to biotic and abiotic stress. Plant Mol Biol 53:383-397

John I, Hackett R, Cooper W, Drake R, Farrell A, Grierson D (1997) Cloning and characterization of tomato leaf senescence-related cDNAs. Plant Mol Biol 33:641-651

Kikuchi K, Ueguchi-Tanaka M, Yoshida KT, Nagato Y, Matsusoka M, Hirano HY (2000) Molecular analysis of the NAC gene family in rice. Mol Gen Genet 262:1047-1051

Kim CM, Je BI, Piao HL, Park SJ, Kim MJ, Park SH, Park JY, Park SH, Lee EK, Chon NS, Won YJ, Lee GH, Nam MH, Yun DW, Lee MC, Cha YS, Lee KH, Eun MY, Han CD (2002) Reprogramming of the activity of the activator/dissociation transposon family during plant regeneration in rice. Mol Cells 14:231-237

Kim CM, Piao HL, Park SJ, Chon NS, Je BI, Sun BY, Park SH, Park JY, Lee EJ, Kim MJ, Chung WS, Lee KH, Lee YS, Lee JJ, Won YJ, Lee GH, Nam MH, Cha YS, Yun DW, Eun MY, Han CD (2004) Rapid, large-scale generation of *Ds* transposant lines and analysis of the *Ds* insertion sites in ride. Plant J 39:252-263

Liu YG, Whittier RF (1995) Thermal asymmetric interlaced PCR: automatable amplification and sequencing of insert end fragments from P1 and YAC clones for chromosome walking. Genomics 25:674-681

Murashige T, Skoog F (1962) A revised medium for rapid growth and bioassays with tobacco tissues. Physiol Plant 15:473-497

Ooka H, Satoh K, Doi K, Nagata T, Otomo Y, Murakami K (2003) Comprehensive analysis of NAC family genes in *Oryza sativa* and *Arabidopsis thaliana*. DNA Res 10: 239-247

Quesada V, Ponce MR, Micol JL (2000) Genetic analysis of salt-tolerant mutants in *Arabidopsis thaliana.* Genetics 154:421-436

Rabbani MA, Maruyama K, Abe H, Khan MA, Katsura K, Ito Y (2003) Monitoring expression profiles of rice genes under cold, drought, and high-salinity stresses and abscisic acid application using cDNA microarray and RNA gel-blot analyses. Plant Physiol 133:1755-1767

Ren T, Qu F, Morris TJ (2000) HRT gene function requires interaction between a NAC protein and viral capsid protein to confer resistance to turnip crinkle virus. Plant Cell 12:1917-1926

Ruiz-Medrano R, Xoconostle-Cazares B, Lucas WJ (1999) Phloem long-distance transport of CmNACP mRNA: Implications for supracellular regulation in plants. Development 126:4405-4419

Sablowski RW, Meyerowitz EM (1998) A homolog of NO APICAL MERISTEM is an immediate target of the floral homeotic genes APETALA3/PISTILLATA. Cell 92:93-103

Saleki R, Young PG, Lefebvre DD (1993) Mutants of *Arabidopsis thaliana* capable of germination under saline conditions. Plant Physiology 101:839-845

Sasaki T, Matsumoto T, Yamamoto K, Sakata K, Baba T, Katayose Y, Wu J, Niimura Y, Cheng Z, Nagamura Y (2002) The genome sequence and structure of rice chromosome 1. Nature 420:312-316

Seki M, Narusaka M, Ishida J (2002*b*) Monitoring the expression profiles of 7000 *Arabidopsis* genes under drought, cold and high-salinity stresses using a full-length cDNA microarray. The Plant Journal 31:279-292

Selth LA, Dogra SC, Rasheed MS, Healy H, Randles JW, Rezaian MA (2005) A NAC domain protein interacts with Tomato leaf curl virus replication accessory protein and enhances viral replication. Plant Cell 17:311-325

Shi R, Pryor JD (2000) Temperature dependence of membrane sealing following transection in mammalian spinal cord axons. Neuroscince 98:157-166

Shinozaki K, Yamaguchi-Shinozaki K, Seki M (2003) Regulatory network of gene expression in the drought and cold stress responses. Current Opinion in Plant Biology 6:410-417

Souer E, van Houwelingen A, Kloos D, Mol J, Koes R (1996) The no apical meristem gene of Petunia is required for pattern formation in embryos and flowers and is expressed at meristem and primordia boundaries. Cell 85:159-170

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

Tsugane K, Kobayashi K, Niwa Y, Ohba Y, Wada K, Kobayashi H (1999) A recessive *Arabidopsis* mutant that grows photoautotrophically under salt stress shows enhanced active oxygen detoxification. Plant Cell 11:1195-1206

Tsugeki R, Kochieva EZ, Fedoroff NV (1996) A transposon insertion in the *Arabidopsis* SSR16 gene causes an embryo-defective lethal mutation. Plant J 10:479-489

Weir I, Lu J, Cook H, Causier B, Schwarz-Sommer Z, Davies B (2004) CUPULIFORMIS establishes lateral organ boundaries

in Antirrhinum. Development 131:915-922

Werner JE, Finkelstein RR (1995) *Arabidopsis* mutants with reduced response to NaCl and osmotic stress. Physiol Plant 93:659-666

Wu SJ, Ding L, Zhu JK (1996) SOS1, a genetic locus essential for salt tolerance and potassium acquisition. Plant Cell 8:617-627

Xie Q, Frugis G, Colgan D, Chua NH (2000) Arabidopsis NAC1 transduces auxin signal downstream of TIR1 to promote lateral root development. Genes Dev 14:3024-3036

Xiong L, Schumaker KS, Zhu JK (2002) Cell signaling during cold, drought, and salt stress. Plant Cell 14:165-183

Yamaguchi-Shinozaki K, Shinozaki K (2006) Transcriptional regulatory networks in cellular responses and tolerance to dehydration and cold stresses. Annu Rev Plant Biol 57: 781-803

Zhu JK (2000) Genetic Analysis of Plant Salt Tolerance Using *Arabidopsis*. Plant Physiology 124:941-948