Theor Appl Genet (2000) 100:782–788 

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# Differential accumulation of the S-adenosylmethionine decarboxylase transcript in rice seedlings in response to salt and drought stresses

Received: 7 July 1999 / Accepted: 29 July 1997

**Abstract** Differences in gene expression between salinity-stressed and normally grown rice seedlings were compared by using the differential display (DD) technique. One DD-derived cDNA clone was characterized as a partial sequence of the rice S-adenosylmethionine decarboxylase (SAMDC) gene by sequence analysis and a homology search of GenBank databases. The full-length cDNA for the rice SAMDC gene, designated *SAMDC1*, was further isolated by the RT-PCR approach and was found to be different from another rice SAMDC gene released in GenBank. Comparison of the deduced polypeptide of *SAMDC1* with SAMDC proteins from other plant species revealed several homologous regions, in particular the conserved proenzyme cleavage site and the putative PEST domain. Southern blot analysis indicated that the *SAMDC1* gene was present as a single-copy sequence in the rice genome. Northern hybridization showed that the transcript of *SAMDC1* was differentially accumulated in rice seedlings in response to salinity, drought and exogenous abscisic acid (ABA) stresses. Furthermore, levels of the *SAMDC1* transcript under saline conditions were compared between a salt-tolerant *japonica* rice variety, Lansheng, and a salt-sensitive *japonica* rice variety, 77–170. It was observed that elevation in the level of the *SAMDC1* transcript occurred earlier in Lansheng than in 77–170 when both were affected by salinity stress. In addition, relative to the control, higher levels of the *SAMDC1* transcript were detected in Lansheng under low salt conditions or salt-stressed for shorter times, and also in 77–170 under high salt conditions or salt-stressed for prolonged times. The results suggest that expression of the *SAMDC1* gene in seedlings is positively correlated with the salt tolerance of rice.

Communicated by H.F. Linskens

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**Key words** Rice (*Oryza sativa* L.) · Differential display · S-adenosylmethionine decarboxylase · Salinity stress · Drought stress

## Introduction

Plants respond to many types of environmental stresses. Among these, osmotic stress, particularly that due to salt and drought stresses, is the most serious problem that limits plant growth and crop production in agriculture. When plants are stressed by environmental factors, a series of physiological and biochemical changes occur. These changes include a decrease in shoot water content, the accumulation of some compatible osmolytes such as sugars, proline and glycine betaine, changes in protein synthesis and gene expression, etc.

Polyamines (PAs) are known to play important roles in the regulation of plant growth and development. The differential accumulation of PAs in different plant species in response to various environmental stresses such as acid stress (Young et al. 1983), osmotic stress (Flores and Galston 1982, 1984; Galiba et al. 1993; Turner and Steward 1986) and salinity stress (Basu and Ghosh 1991; Basu et al. 1988; Friedman et al. 1989; Krishnamurthy and Bhagwat 1989) has been observed, suggesting that PAs also play an essential role in the responses of plants to adverse environments. The polyamine biosynthesis pathways have been well-established. In plants, putrescine, which plays a pivotal role in these pathways, can be produced directly from ornithine by ornithine decarboxylase (ODC, EC 4.1.1.17) or indirectly from arginine by arginine decarboxylase (ADC, EC 4.1.1.19). Putrescine then is converted into spermidine and spermine by adding propylamino groups from decarboxylated S-adenosylmethionine (dcSAM), which is produced from S-adenosylmethionine (SAM) by the action of S-adenosylmethionine decarboxylase (SAMDC, EC 4.1.1.50) (Walden et al. 1997). SAMDC is probably the rate-limiting enzyme in polyamine biosynthesis pathways because the level of dcSAM in living organisms is very low and, moreover, the SAMDC protein

has a relatively short half-life of about 1–2 h (Tabor and Tabor 1984).

Differential display is a powerful tool for the isolation of plant genes regulated by salt stress (Zhang and Chen 1996; Nemoto et al. 1999). In the study presented here, differential display was performed to isolate salt-responsive genes from rice. A salt-inducible partial cDNA clone representing the 3' sequence of the rice SAMDC gene was obtained, and the full-length cDNA of this gene was isolated. The deduced amino acid sequence of this gene was different from that of another rice SAMDC gene found in the conserved proenzyme cleavage site. We also performed Northern blot analysis to investigate the expression of the SAMDC gene in rice seedlings in response to salinity, drought and exogenous abscisic acid (ABA) stresses. Moreover, levels of SAMDC transcript under salinity stress condition between salt-tolerant and salt-sensitive rice varieties were also compared.

## Materials and Methods

### Plant materials, growth conditions and stress treatments

Seeds of rice (*Oryza sativa* L. ssp. *indica* cv. Zhaiyeqing8) were germinated at 37°C for 3 days and grown hydroponically at 26°C at a photoperiod of 12 h. At the three-leaf stage, rice seedlings were grown for 3 days either in solutions containing 1% (about 171 m*M*) NaCl or in water. Total RNA was extracted from the seedlings and used for differential display analysis.

Rice seedlings at the three-leaf stage were transferred into solutions containing 171 mM NaCl, 20 μ*M* ABA, and 15% PEG6000 for the salinity, ABA and drought stress treatments, respectively. A salt-tolerant *japonica* rice variety, Lansheng, and a salt-sensitive *japonica* rice variety, 77–170, were also grown and treated with different concentrations of NaCl or with NaCl at the same concentration for different time courses. The harvested seedlings were quickly frozen in liquid nitrogen and stored at –70°C for RNA extraction.

### Differential display (DD)

Total RNA was extracted from rice shoots using the guanidinium isocyanate/acidic phenol method (Zhang et al.1995). First-strand cDNAs were synthesized from 0.4 μg total RNA (pre-treated with DNase I) for 50 min at 42°C in a 40-μl reaction volume with M-MLV reverse transcriptase (Promega). Differential display was performed essentially as described by Liang and Pardee (1992). Polymerase chain reaction (PCR) analysis was carried out in a total volume of 25 μl containing of 2 μl of first-strand cDNAs, 2.5 μ*M* $dT_{12}MN$, 0.5 μ*M* arbitrary primer (Operon), 1 × PCR buffer, 2 μ*M* dNTPs, 185 Bq α-[$^{32}$P]-dCTP (Amersham) and 1.0 U *Taq* DNA polymerase. The amplification profile was 3 min at 94°C for pre-denaturation; 40 cycles of 1 min at 94°C, 2 min at 40°C, 1 min at 72°C, a final extension for 5 min at 72°C. The amplified products were separated on a 6% non-denaturing polyacrylamide gel and exposed to X-film directly. The cDNA fragments showing signal differences between salt-stressed and control seedlings were retrieved from the sequencing gel and eluted by boiling for 10 min in 100 μl water; 5 μl was used directly as templates for PCR reamplification. The reamplification conditions were the same as those in the initial DD-PCR reaction except that the dNTP concentration was increased to 20 μ*M* and no isotope was added. The reamplified cDNA fragments were recovered from the agarose gel and cloned into pGEM-T easy vector (Promega) according to the manufacturer's instruction.

### Reverse transcription PCR (RT-PCR)-based cDNA cloning

The full-length cDNA for the rice SAMDC gene was isolated using a PCR-based approach. Based on the 5' untranslated sequence of the rice SAMDC gene released in GenBank (accession no. Y07766), a specific sense primer, 5'-GCTTCCTGATAATCGAACCAG-3', was synthesized and used to perform RT-PCR amplification in combination with a *SAMDC1*-specific anti-sense primer, 5'-CGCAGCTGACCACCTAGAGC-3'. The PCR conditions were a pre-denaturation of 3 min at 94°C, 35 cycles of 1 min at 94°C, 1.5 min at 55°C, 1.5 min at 72°C; an extension for 10 min at 72°C. The amplified cDNA fragment was purified and cloned for sequencing.

### RNA gel blot analysis

Thirty micrograms of total RNA was denatured, separated on a 1.2% fomaldehyde agarose gel and blotted onto nylon membranes (Hybond $N^+$, Amersham) in 20 × SSC solution. The RNA was fixed on the membrane by exposure to UV light for 3 min. Northern hybridization was carried out overnight at 65°C by using an α-[$^{32}$P]-dCTP-labeled, 600-bp 3' sequence of *SAMDC1* as a probe. Filters were washed with 2 × SSC, 0.1% SDS and 1 × SSC, 0.1% SDS for 15 min at 42°C, respectively, then washed with 1 × SSC, 0.1% SDS for 15 min at 55°C. After stripping the probes, we re-probed the same blots with the 18s rDNA gene. The mRNA levels were quantified using the Imaging DensitoMeter (Model GS-670 Bio-Rad). The resulting values were normalized with those obtained from 18s rDNA hybridization.

### Genomic Southern blot analysis

DNA extraction and Southern blot analysis were carried out as described previously (Zhang et al. 1995). Hybridization was carried out for 16 h at 65°C with a α-[$^{32}$P]-dCTP-labeled, 600-bp 3' sequence of *SAMDC1* as a probe. The membrane was washed with 2 × SSC, 0.1% SDS; 1 × SSC, 0.1% SDS and 0.5 × SSC, 0.1% SDS for 15 min at 65°C, respectively.

### DNA sequencing and data analysis

DNA sequences were determined using the Taq Dye Primer Cycle Sequencing Kit (Amersham) and ABI 373A automatic sequencer. The nucleotide and amino acid sequences were compared with those released in GenBank databases by using the GAPPED BLAST analysis program. The full-length sequence of *SAMDC1* has been deposited in GenBank databases under the accession number AF067194.

## Results

### Differential display

Differential display was performed to compare differences in gene expression between salt-stressed and normally grown rice seedlings. Five 3' anchor primers ($dT_{12}GG$, $dT_{12}CG$, $dT_{12}AG$, $dT_{12}GC$ and $dT_{12}CC$) and ten arbitrary primers (10 mer) were screened in DD-PCR reactions. Of the 3000 cDNA fragments displayed on the sequencing gel, 31 were shown to be differentially expressed under conditions of salinity stress. One positive clone, which was amplified by primer pair $dT_{12}CC$/OPA01 (shown in Fig. 1), was chosen for further analysis. Sequence analysis of this clone revealed that

it was 600 bp in length and was homologous to the SAMDC sequences of rice (Y07766) and Tritordeum (X83881) in the GenBank. These observations indicate that the DD-derived partial cDNA fragment indeed represents the SAMDC gene of rice.

## Characterization of the SAMDC gene of rice

To obtain more sequence information on the rice SAMDC gene, we performed RT-PCR to amplify the full-length cDNA of the SAMDC gene (See Materials and methods). Sequence analysis of the PCR product revealed that it is different from the rice SAMDC gene (Y07766), particularly in the conserved proenzyme cleavage site. The amino acid sequences from residues 76 to 85 in the SAMDC sequence obtained in the present study are SESSLFIYSD, however, amino acid sequences of LSPACLSILI were found in the same region of another rice SAMDC sequence (Y07766). Beyond this, there are 15 nucleotide substitutions, resulting in 12 synonymous amino acid substitutions and 3 missense amino acid substitutions in the coding region. Moreover, the 3' untranslated sequence of the SAMDC gene obtained in present study is 22 bp shorter than that of another rice SAMDC gene, and the poly(A) addition occur at a different site. These observations suggest that the cDNA sequence obtained in present study represents an uncharacterized SAMDC gene of rice, and we tentatively named it *SAMDC1*. The reconstituted full-length cDNA of *SAMDC1* is 1560 bp in length consisting of a 90-bp 5' untranslated region, a complete open reading frame of 1,194 bp encoding a polypeptide of 398 amino acids, followed by a 3' untranslated sequence of 273 bp. A homology search of GenBank demonstrated that the SAMDC1 protein was homologous to SAMDC proteins from plants, yeast and mammals. Alignment of the deduced polypeptide sequence of *SAMDC1* with SAMDC sequences from Tritordeum (Dresselhaus et al. 1996), potato (MadArif et al. 1994) and spinach (Bolle et al. 1995) illustrated the presence of several highly conserved regions (Fig. 2). These regions included an amino acid sequence of LSESSLF, which is a putative proenzyme cleavage site, from residues 75 to 81 in *SAMDC1* and a putative PEST domain of TIHVTPEDGFSYASYE from residues 254 to 269 in *SAMDC1*. The PEST domain is considered to be associated with the rapid SAMDC protein turnover (Rogers et al. 1986).

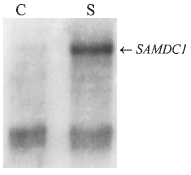


**Fig. 1** Differential display of total RNA from normally grown (*C*) and salt-stressed (*S*) Zhaiyeqing8 seedlings. The anchor primer $dT_{12}CC$ and the arbitrary primer OPA01 were used for DD-PCR amplification. *Arrowhead* The differentially expressed partial cDNA fragment of *SAMDC1*

**Fig. 2** Comparison of the deduced amino acid sequence of *SAMDC1* with SAMDC sequences of Tritordeum, potato and spinach. *Gaps* are introduced to maximize similarities. *Dots* indicate the residues identical to those of SAMDC1. The conserved proenzyme cleavage site and PEST domains are *boxed*, and the *black triangle* indicates the cleavage site

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RICE          MGVLSAADPPPVSAIGFEGYEKRLEITFSEAPVFADPDGRGLRALSRAQIDSVLDLARCTIVS   63
TRITORDEUM    .-------AA.....................SI....H.........................   56
POTATO        .-----EMDL.........F......S.V.PGL....N.K...S..K..L.EI.GP.E....D   58
SPINACH       .-------AI--.......F........F.PSI.V..E.K.....CK..L.EI.GP.E....D   54

RICE          ELSNKDFDSYVLSESSLFIYSDKIVIKTCGTTKLLLTIPRILELAEGLSMPLAAVKYSRGMFI  126
TRITORDEUM    ..........|.....|...Q.........M.............E.C.............   119
POTATO        N...DYV...|.....|V..Y..I..........A..P..R...T..LKVQD.R.T..S..   121
SPINACH       S.A.ESV...|.....|..AY..I..........RA..P..R..GK..LDVKS.R.T..S..   117

RICE          FPSAQPAPHRSFSEEVAVLNRYFGHLKSGGNAYVI-GDPAKPGQKWHIYYAT---QHPEQPMV  185
TRITORDEUM    ..G.............D.........N........-..............---EQ......   178
POTATO        ..G..SF...H.........DG...K.AA.SK.-..M.S.D.T-....V.S.SAGSVQSND.VY  182
SPINACH       ..G..SYA...........DG...K.AA.SK.-FVM......-....V.S.SAETISF.E.VY  178

RICE          TLEMCMTGLDKEKASVFFKTSADGHTSCAKEMTKLSGISDIIPEMEICDFDFEPCGYSMNAIH  248
TRITORDEUM    ...........T........H....V.....................V..............N  241
POTATO        ..........R......Y..----EE.S.AH..VR...RK.L.KS.....E.........S.E  241
SPINACH       .........K.........S----QSPN.AV..ES...RK.L.DSK................E  237

RICE          GLAFS|TIHVTPEDGFSYASYE|VVGFDASTLAYGDLVKRVLRCFGPSEFSVAVTIFGGHGHAGT  311
TRITORDEUM    .S...|.................|.Q.M...A.....I.....................R...A.  304
POTATO        .A.V.|...I......T...F.|S..YNPK.MEL.P..E...A..E.A.....LHADVATKLLER  304
SPINACH       .P.I.|...I..........F.|A..Y.LKKTDLNQ..E...A..E.....I.IHAEIAANSMEH  300

RICE          WAKELNADAYKCNNMVEQELPCGGLLIYQSFDATEDVPVAVGSPKSVLHCFEAENMVNPAPVK  374
TRITORDEUM    .G.K.D.E..D...V.........V......A.N.ELA.SA...R..F....NVESGH.-L..  366
POTATO        ICSVDVKG-.SLAEWSPE.FGE..SIV..K.TR.PYCESPKSVL.----GCWKEEEKEGKE    360
SPINACH       NCYVNVNG-.SREEGGIE-GF.AASVF..K.CKASTGFGATNKP.PALK.CWKEDKFEEEKDY  361

RICE          EGKLGNLLPW--GEDALEENDG-VFDE                                      398
TRITORDEUM    ....A...A.RAE.ES...GT.ALLC.                                      393
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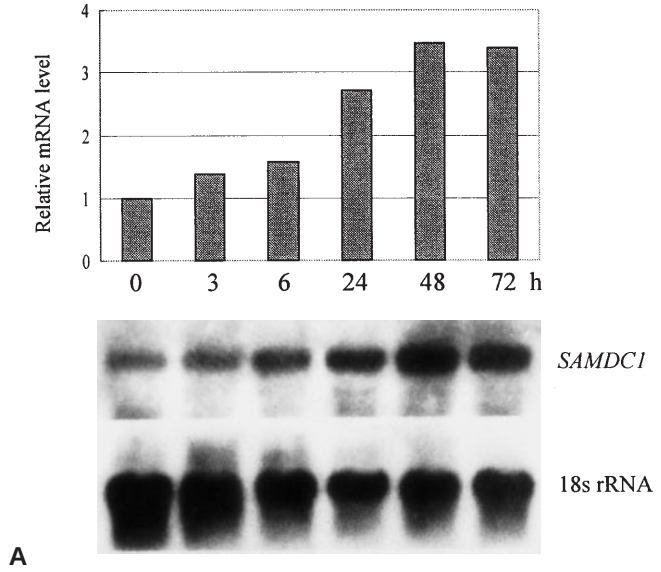


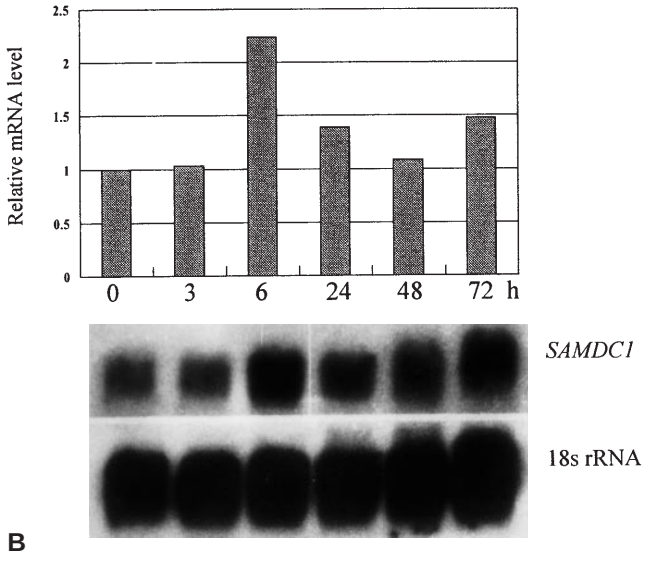


**Fig. 3A, B** Differential expression of *SAMDC1* gene in rice seedlings in response to salinity (**A**) and drought (**B**) stresses. Total RNA was extracted from rice seedlings treated with 1% NaCl or 15% PEG6000 for different time courses. The same blots were stripped of probes and re-hybridized with 18s rDNA gene. The mRNA levels were quantified and the resulting values were normalized with those obtained from 18 s rDNA hybridization. The *column size* represents the relative mRNA level generated by comparing the normalized values of each lane with that of the control

## Southern blot analysis

Southern blot analysis was carried out to investigate the organization of *SAMDC1* in the rice genome. Only one restriction fragment of various sizes resulting from different restriction enzyme digestion was detected under high-stringency hybridization conditions (data not shown), indicating that the *SAMDC1* gene was present as a single-copy sequence in the rice genome.

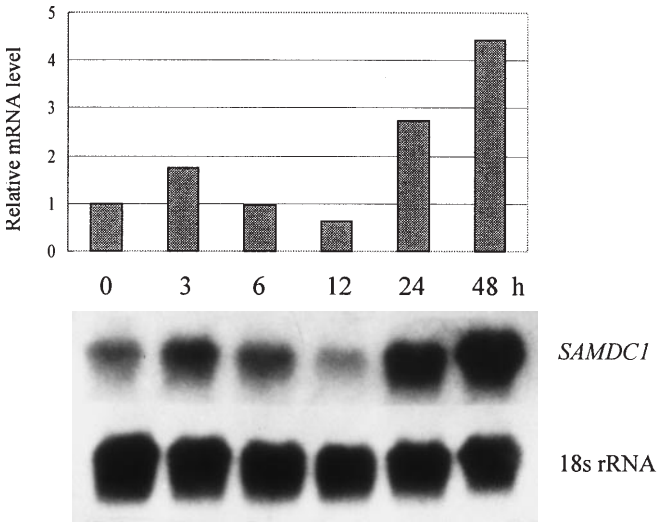


**Fig. 4** Time-course RNA gel blot analysis of *SAMDC1* transcript in response to ABA in rice shoots. Total RNA was extracted from rice seedlings stressed with 20 μ*M* ABA at the indicated time points. Northern blot analysis and normalization of the hybridization signal were the same as that described in **Fig. 3**

## Analysis of the effects of salinity, drought, and exogenous ABA stresses on the expression of *SAMDC1*

Northern blot analysis was performed to investigate the effects of various environmental stresses on the expression of *SAMDC1* in rice seedlings. Salinity stress caused a steady accumulation of the *SAMDC1* transcript in rice shoots, and its level reached a peak at 48 h, and then slightly declined (Fig. 3A). Drought stress also induced the expression of *SAMDC1*, and the peak level of the transcript was detected at 6 h after stress (Fig. 3B).

ABA is implicated in plant stress responses. Exogenous application of ABA also affected the expression of *SAMDC1* in rice shoots, but a rather sophisticated pattern was detected. As shown in Fig. 4, induction of the *SAMDC1* gene was detected at 3 h and 24–48 h after application of the stress. In contrast, repression of the gene was observed at 12 h after ABA treatment, resulting in a decrease in the level of the *SAMDC1* transcript relative to the control.

## Comparison of levels of the *SAMDC1* transcript under salinity conditions between salt-tolerant and salt-sensitive rice varieties

To address the function of *SAMDC1* in the tolerance of rice to salinity stress, we performed Northern blot analysis to compare levels of the *SAMDC1* transcript under saline conditions in a salt-tolerant *japonica* rice variety, Lansheng, and a salt-sensitive *japonica* rice variety, 77–170. As shown in Fig. 5, accumulation of the *SAMDC1* transcript in Lansheng was detected 1 h after

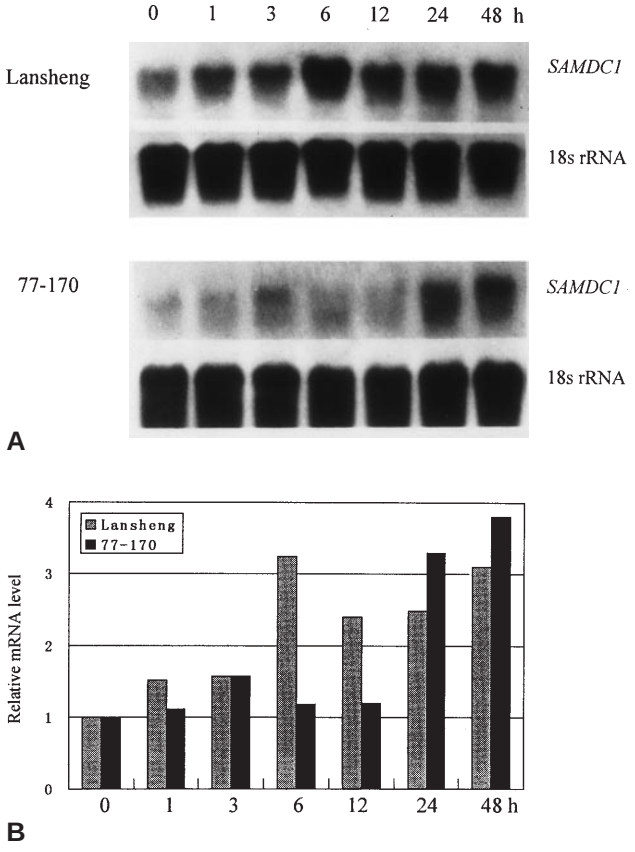


**Fig. 5A, B** Comparison of levels of the *SAMDC1* transcript between salt-tolerant and salt-sensitive rice varieties under saline conditions. Total RNA was extracted from Lansheng and 77–170 seedlings stressed with 1% NaCl for different time courses. Northern blot analysis (**A**), and normalization of the hybridization signal (**B**) were the same as that described in **Fig. 3**

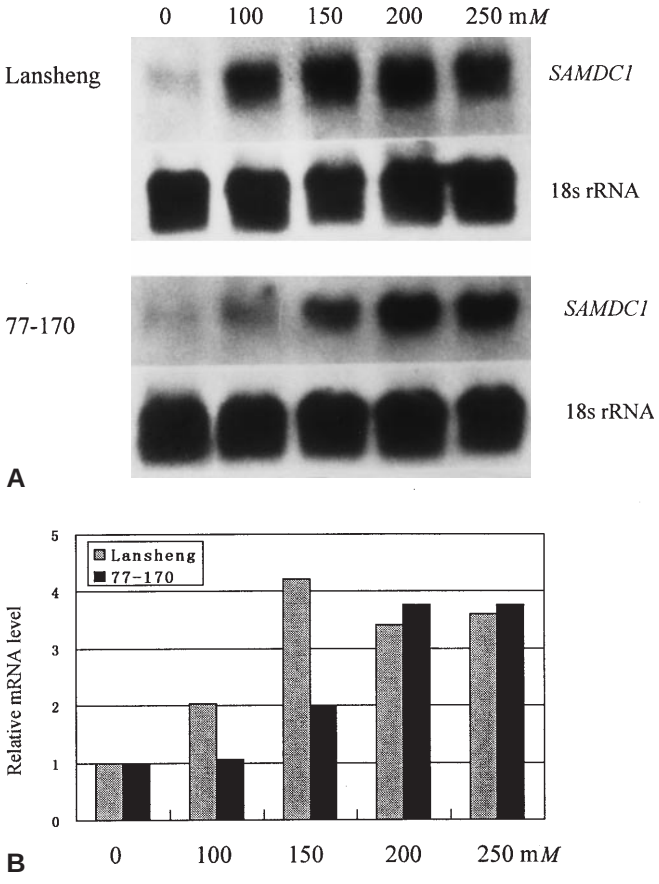


**Fig. 6A, B** Comparison of levels of the *SAMDC1* transcript between salt-tolerant and salt-sensitive rice varieties under saline conditions. Total RNA was extracted from Lansheng and 77–170 seedlings stressed for 24 h with different concentrations of NaCl. Northern blot hybridization (**A**), and normalization of the hybridization signal (**B**) were the same as that described in **Fig. 3**

salt stress was applied, it reached a peak at 6 h and then it slightly declined. Conversely, the level of the *SAMDC1* transcript in 77–170 was slightly elevated in slightly less than 12 h, and reached its peak at 48 h after salt stress. These results indicate that induction of the *SAMDC1* transcript in 77–170 was slower than that in Lansheng. In addition, *SAMDC1* transcript accumulation in 77–170 was also lower than that in Lansheng as affected by NaCl at low concentrations (Fig. 6). Treatments with 100 m*M* and 150 m*M* NaCl were effective in inducing a twofold and fourfold accumulation of the *SAMDC1* transcript in Lansheng, respectively, but only slight induction of the gene or only a twofold accumulation of the *SAMDC1* transcript was detected in 77–170 seedlings treated with 100 m*M* or 150 m*M* NaCl (Fig. 6). However, in rice seedlings exposed to higher concentrations of NaCl (200 m*M* and 250 m*M*) or salt-stressed for prolonged times (24 h and 48 h), the level of *SAMDC1* transcript accumulation in 77–170 was higher than that in Lansheng (Figs. 5, 6).

## Discussion

Several genes encoding SAMDC have been cloned and characterized from *E. coli* (Tabor and Tabor 1987), yeast (Kashiwagi et al. 1990), mammals (Pajunen et al. 1988) and plant species such as potato (MadArif et al. 1994), spinach (Bolle et al. 1995), *Catharanthus roseus* (Schröder and Schröder 1995), carnation (Lee et al. 1997) and Tritordeum (Dresselhaus et al. 1996). In addition, full-length or partial sequences of the SAMDC gene from *Arabidopsis thaliana* (Accession no. U63633, Y07765), *Oryza sativa* (C98665, D23921 and Y07766), *Zea mays* (Y07767), *Nicotiana tabacum* (U91924, AF033100) and *Triticum aestivum* (AF117660) have been deposited in GenBank databases, but the function of these sequences has not been further characterized. In the present study, a full-length cDNA (1,560 bp) that encodes S-adenosylmethionine decarboxylase (SAMDC) was isolated from *indica* rice using differential display and subsequent RT-PCR approaches. The deduced polypeptide sequence of *SAMDC1* is different from another rice SAMDC sequence (Y07766), particularly in the conserved proen-

zyme cleavage site, indicating that *SAMDC1* represents a new sequence in the rice genome.

Alignment of the predicted amino acid sequence of *SAMDC1* with SAMDC proteins from other plants identified several conserved regions, particularly the proenzyme cleavage site and the putative PEST domains (Fig. 2), suggesting the structural and functional similarities of SAMDC proteins in the plant kingdom. The cleavage of the SAMDC proenzyme, resulting in the formation of a small β-chain (N-terminal part) and a larger α-chain (C-terminal part), has already been demonstrated (Kashiwagi et al. 1990; Lee et al. 1997; Pajunen et al. 1988; Schröder and Schröder 1995) and confirmed to be essential for the biological function of the SAMDC enzyme *in vivo* (Schröder and Schröder 1995). As a result of this post-translational modification, a covalently linked pyruvate prosthetic group, which is essential for enzymatic activity, is generated from the N-terminal serine residue at the cleavage site (Kashiwagi et al. 1990; Tabor and Tabor 1987). The PEST domain, first described by Rogers et al. (1986), is a short stretch of amino acids abundant in proline (P), glutamic acid (E), serine (S) and threonine (T) residues. Proteins with half-life shorter than 2 h often contain at least one PEST domain, and removal of the PEST region can stabilize proteins within the cells, indicating that the PEST domain is responsible for a rapid protein turnover.

The accumulation of PAs in response to environmental stresses could be the consequences of the induction of polyamine biosynthesis enzyme-encoding genes or this enzyme activities. Results from our experiments show that expression of the *SAMDC1* gene in rice seedlings is dramatically induced by salinity and drought stresses (Fig. 3), suggesting that regulation at the transcriptional level plays an important role in activation of the SAMDC gene under stress conditions. Although it has been well-documented that PAs are implicated in the responses of plants to abiotic stresses (Reviewed by Flores 1990), the definite function of PAs in stress responses remains unclear. Results from some studies suggest that PAs, particularly spermidine and spermine, are involved in the regulation of gene expression by enhancing the DNA-binding activities of some transcription factors (Gupta et al. 1998; Panagiotidis et al. 1995). On the other hand, PAs are considered to serve as an osmo-protectant in plant cells under water-deficit conditions (Bohnert et al. 1995). Besford et al. (1993) have demonstrated that the exogenous application of spermidine and spermine can maintain the membrane structure in osmotically stressed mesophyll protoplasts of oat leaves.

ABA is known to be implicated in responses of plants to various environmental stresses, and most stress-inducible genes are induced by exogenous ABA treatment. Our results show that expression of the *SAMDC1* gene is also affected by ABA (Fig. 4), suggesting activation of the *SAMDC1* gene through ABA-dependent pathways. However, reduction in the level of the *SAMDC1* transcript 12 h after ABA treatment was observed. It has also been reported that an excess of ABA over jasmonates (JA) reduced *salT* transcript accumulation in rice roots (Moons et al. 1997). The negative effects of ABA on *SAMDC1* transcript accumulation probably occur at the post-transcriptional level by affecting mRNA stability and/or turnover.

Comparison of *SAMDC1* transcript levels in two *japonica* rice varieties differing in salt tolerance showed that the elevation in the level of the *SAMDC1* transcript in the salt-tolerant rice variety was higher than that in salt-sensitive rice variety when both were affected by low concentrations of NaCl stress (Fig. 6). It was also observed that accumulation of the *SAMDC1* transcript in the tolerant variety occurred more quickly than that in the sensitive variety (Fig. 5). These observations suggest that salt-tolerant rice responds more quickly to salinity stress than salt-sensitive rice does, although the mechanisms by which the salt-tolerant rice perceives and transducts the stress signal remain unclear. In addition, it can also be deduced from the Northern data that accumulation of the *SAMDC1* transcript in rice seedlings is positively correlated with the salt tolerance of rice. This conclusion can be evidenced by the results obtained by Krishnamurthy and Bhagwat (1989) that salt-tolerant rice varieties are effective in maintaining higher levels of spermidine and spermine under saline conditions, whereas salt-sensitive rice varieties tend to accumulate more putrescine. On the other hand, relatively higher levels of the *SAMDC1* transcript was detected in the salt-sensitive rice variety under high salt conditions or exposed to prolonged times as compared with that in the salt-tolerant rice variety (Figs. 5, 6). It is possible that under high salt conditions or salt-stressed for prolonged times, the tolerant rice was able to adapt to the detrimental circumstances by other mechanisms such as compartmentation of $Na^+$ and $K^+$, accumulation of high levels of compatible osmolytes, etc. However, the sensitive rice could not make similar adjustments to the salinity environment, thus the high level of spermidine and spermine results from activation of the *SAMDC1* gene could have adaptive value to favor its survival. Therefore, it is necessary to conduct transgene experiments to investigate the function of the *SAMDC1* gene product with respect to salt damage in salt-sensitive rice varieties.

**Acknowledgements** The authors are grateful to Dr. Jin-Song Zhang, Dr. Yu-Jing Li, Mr. Can Xie and Mr. Yi-Guo Shen for technical assistance and valuable discussions. This work was supported by a grant from the High-Technology Development Program of China and a grant from the Major State Basic Research Program of China (G1999011703)

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