The Overexpression of *Cyanidioschyzon merolae* S-adenosylmethionine Synthetase Enhances Salt Tolerance in Transgenic *Arabidopsis thaliana*

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**Summary** High salinity is one of the most serious threats to crop production. The primitive red alga, *Cyanidioschyzon merolae*, inhabits an extreme environment (42°C, pH 2.5, high salt, metal ion). We have utilized the ability of *C. merolae* cells to adapt to 0.3 M sodium salt, as well as information from its fully sequenced genome, to produce salt-tolerant transgenic higher plants. To reveal the mechanisms of high salt tolerance, we analyzed, by RT-PCR, genes that were expressed at high levels after salt stress (0.3 M NaCl). The *C. merolae* S-adenosylmethionine synthetase (CmSAMS) gene that codes for an enzyme in the polyamine biosynthesis pathway was expressed at high levels (4 to 5 expression ratio). Our results are in accordance with our previously reported DNA microarray data. The CmSAMS gene codes for a 393-aa protein contain 3 conserved domains at the N-terminal and a semi-conserved domain at the C-terminal. The particle bombardment method revealed that the recombinant CmSAMS-green fluorescent protein was localized in the cytoplasm and the nuclei of the plant cells. To further investigate tolerance to salt stress, we produced, by *Agrobacterium*-mediated transformation, 4 transgenic *Arabidopsis thaliana* plant lines expressing CmSAMS. Compared to wild-type plants, the CmSAMS transgenic plants were more tolerant to salt stress, clearly defining a role for the CmSAMS gene in conferring salt-stress tolerance.

**Key words** *Cyanidioschyzon merolae*, Hot spring alga, Salt tolerance, Real-time polymerase chain reaction, S-adenosylmethionine synthetase.

The problem of high salt stress is likely to increase due to the potentially catastrophic consequences for the biosphere from global warming. Genetic engineering to produce high salt tolerant plants will play a part in overcoming the drying and desertification. High salinity has been estimated to reduce agricultural productivity on more than 20% of the world’s cultivated land (Botella et al. 2005). Methods to improve the stress resistance and survival of plant seedlings are becoming extremely important for reforestation and ecosystem reestablishment in saline areas.

Several studies have demonstrated the acquisition of high salt tolerance using mutations in flowering plants such as *A. thaliana* and by the transformation of salt-resistant genes from flowering plants that survive in high salt conditions (Yamaguchi-Shinozaki and Shinozaki 1994, Gong et al. 2005). DNA microarrays can be used to monitor changes in the transcript levels of almost all the genes in a specific organism (Watson et al. 1998, DeRisi et al. 1999, Fujiwara et al. 2009). In *A. thaliana* (Seki et al. 2002) and *Synechocystis* (Hihara et al. 2001, Suzuki et al. 2001), such arrays have been used to examine gene expression in response to various kinds of stress. In *Saccharomyces cerevisiae*, transcript expression at high salinity was studied (Yale and Bohnert 2001). As a result, polyamine biosynthetic genes have been identified as being involved in

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environmental stress tolerance. When under drought stress, transgenic rice plants expressing the *Datura stramonium* arginine decarboxylase (adc) gene produced much higher levels of putrescine (Put), resulting in higher levels of spermidine (Spd) and spermine synthesis that ultimately protected the plants from drought (Capell et al. 2004). The introduction of the S-adenosylmethionine decarboxylase (SAMDC) gene has been shown to confer stress tolerance to salt and drought in rice (Roy and Wu 2002), and to salt, drought, and fungal wilts in tobacco (Waie and Rajam 2003). The enhancement of multiple environmental stress tolerances by the exogenous spermidine synthase (SPDS) gene has been reported in *A. thaliana* (Kasukabe et al. 2004) and in *Ipomoea batatas* (Kasukabe et al. 2006). These results indicate that the genetic manipulation of Spd could be an effective strategy for simultaneously conferring multiple stress tolerance. Such a strategy is of practical importance since plants often suffer several kinds of environmental stress at the same time.

Our aim, in this study, is to identify the most important gene in the polyamine synthesis pathway that confers salt-stress tolerance. The primitive red alga, *C. merolae*, has the ability to grow in high salt environments (Sakajiri et al. 2008), and all of the 3 genome compartments of the alga (cell nucleus, 16,546,747 bp; mitochondrion, 32,211 bp and plastid, 149,987 bp) have been fully sequenced (Matsuzaki et al. 2004), making it an ideal model to study the mechanism of salt-stress tolerance. Using microarrays to examine specific gene expression in high-salt conditions, we have shown that CmSAMS is the most highly expressed enzyme in the polyamine biosynthesis pathway (Sakajiri et al. 2008). In the present study, we have re-examined the microarray data by RT-PCR and produced transgenic salt-tolerant *A. thaliana* using the expressed genes that are involved in the adaptation of *C. merolae* to salt stress.

Materials and methods

*Algal culture and salt stress treatments*

*C. merolae* cells were grown at 42°C in Allen’s photoautotrophic medium under continuous white fluorescent lamp. Three hundred milliliters of cells were shaken in a 500-ml flask under air. For stress treatment, 300 ml aliquots were centrifuged at 3000 rpm for 5 min, the Allen’s medium was discarded and Allen’s medium containing 0, 0.15, 0.175 and 0.3 M NaCl was added to make up the samples.

*RNA preparation*

Nucleic acid isolation buffer (50 mM Tris-HCl pH 7.6, 0.1 M EDTA, 0.3 M NaCl, 4% SDS, 2% N-lauroylsarcosine sodium salt), pre-warmed to 60°C, was added to the frozen cell pellets. The lysate was added to an equal volume of PCI (phenol : chloroform : isoamylalcohol=25 : 24 : 1). The aqueous phase was recovered by centrifuging at 15,000×g for 5 min at 4°C and re-extracted using PCI. Total nucleic acid was precipitated by adding an equal volume of isopropanol and recovered by centrifugation at 15,000×g for 15 min at 4°C. The pellet was melted in DNase I solution (0.1 unit/μl DNase I, RNase Free; Roshe, 0.4 unit/μl RNase Inhibitor; Sigma, 400 mM Tris-HCl, 100 mM NaCl, 60 mM MgCl2, 10 mM CaCl2, pH 7.9, 10 mM DTT) and incubated for 45 min at 37°C. Total RNA was precipitated by adding an equal volume of isopropanol and recovered by centrifugation at 15,000×g for 15 min at 4°C.

*Quantitative real-time reverse transcription-PCR and RT-PCR*

To confirm the reliability of microarray hybridization, quantitative real-time RT-PCR was carried out using the One Step RNA PCR kit (AMV; TaKaRa). The partial nucleotide sequences of each gene were amplified by RT-PCR with the following primers: for SAMS 5’-GCCCTGCGCG-ATCA-3’ and 5’-TGGCTACTTGGCCTTCAG-3’, for SAMDC 5’-AAGTGCTCCCGATGGTA-
TG-3’ and 5’-CTGCTGCCCATCCAGTGAT-3’, for SPDS 5’-CGAGTGGCCAGATAGGATTCC-3’ and 5’-GCCGTTGCGCCATACC-3’, for ACC 5’-TGCAGTCGCCAGCGATAA-3’ and 5’-GCCTCGACAGCAGCATCT-3’, for ADC 5’-GCTGCTGACCTCACCAACCT-3’ and 5’-GCTGCGATACTGCGCACAT-3’. For real-time PCR, 2 mg total RNA was used for RT with SuperScripts reverse transcriptase. The cDNA samples were diluted to 2 and 8 ng/ml. Triplicate quantitative assays were performed on 1 ml of each cDNA dilution with the SYBR Green Master mix and an ABI 7900 sequence detection system according to the manufacturer’s protocol (Applied Biosystems). The relative quantification method (Delta-Delta CT) was used to evaluate quantitative variation between the replicates examined. Real-time RT-PCRs of the genes encoding the enzymes (ACC, 1-aminocyclopropane-1-carboxylic acid; ADC, arginine decarboxylase; SPDS, spermidine synthase, SPMS, spermine synthase, SAMDC, S-adenosylmethionine decarboxylase and CmSAMS) that exist in the pathway of spermidine and spermine synthesis were carried out using conventional methods (Fujiwara et al. 2009).

One microgram of total RNA was used as template for reverse transcription in each reaction. The amplification reaction was repeated using a GAPDH gene as the template RNA loading control. RT-PCR reactions were repeated 5 times.

**Phylogenetic analysis of CmSAMS**

The SAM sequences were obtained from the *C. merolae* Genome Project (http://merolae.biol.s.u-tokyo.ac.jp/), GenBank (http://www.ncbi.nlm.nih.gov/Genbank/) and JGI (http://genome.jgi-psf.org/). The sequences were automatically aligned using CLUSTAL X version 1.81 (Thompson et al. 1997; ftp://ftp-igbmc.u-strasbg.fr/pub/ClustalX/). For the phylogenetic analyses, ambiguously aligned regions were rearranged manually or deleted using the BioEdit Sequence Alignment Editor version 7.0.9 (http://www.mbio.ncsu.edu/BioEdit/bioedit.html). Edited sequences (containing gaps) that were 440 amino acids long were selected and used in the analyses. Maximum likelihood (ML) analyses were performed using PROML in PHYLIP version 3.6a (Felsenstein 2002) under the JTT model (among-site rate variation model with 8 rate categories) with global rearrangements and one-jumble options. Bootstrap analyses of ML and maximum parsimony (MP) were performed using the PHYLIP package (PROML for ML, PROTPARS for MP). Datasets of 100 replicates for ML and 1000 replicates for MP were produced by SEQBOOT. Bootstrap values were calculated using CONSENSE. Bootstrap analysis of neighbor joining (NJ) was performed by CLUSTAL X.

**Localization of CmSAMS-GFP as shown by particle bombardment**

To examine the localization of CmSAMS, CmSAMS-GFP was constructed and GFP vector, mitochondria (mt)-targeted GFP and chloroplast (cp)-targeted GFP constructs (Chiu et al. 1996, Niwa et al. 1999) were used as controls (Fig. 3). The full length CmSAMS coding region was cloned into the pPZP221 binary vector that contained the aac1 gene encoding gentamycin resistance (Hajdukiewicz et al. 1994), the CaMV35S promoter, sGFP and the NOS terminator (Niwa et al. 1999). The constructs were introduced into onion epidermis cells using the particle-bombardment method and the recombinant proteins were transiently expressed in the transformed cells. Localization of the controls and of CmSAMS-GFP was examined by fluorescence microscopic imaging after particle bombardment according to the method described previously (Hirooka et al. 2009).

**Fluorescence microscopy**

The localization of GFP was examined by light microscope and fluorescence microscopic imaging (under green excitation) after particle bombardment according to Hirooka et al. (2009).
Microscopic images of wild type and CmSAMS 1 cotyledons cultured in 0.15 M NaCl and 0.175 M NaCl were examined by light microscope and fluorescence microscopic imaging (under green excitation) according to Hirooka et al. (2009).

**Transformation of CmSAMS and AtSAMS into A. thaliana**

The full-length CmSAMS and AtSAMS coding region were cloned into the pPZP221 binary vector that contained the aac1 gene encoding gentamycin resistance (Hajdukiewicz et al. 1994), the CaMV35S promoter and the NOS terminator sequences. The constructs were introduced into A. thaliana plants by the floral dip method using Agrobacterium tumefaciens strain GV3101 containing the helper plasmid pMP90 (Clough and Bent 1998). The A. thaliana plants were grown at 23°C in 10-cm pots filled with a 1:1 mixture of perlite and vermiculite under a long-day photoperiod (16-h light/8-h dark). A growth chamber (CLH-301; TOMY, Tokyo, Japan) equipped with white fluorescent lamps (FL40SW, NEC 129 Co., Ltd., Tokyo, Japan) was used. Transgenic A. thaliana were selected on MS agar media containing 1% agar, 1% sucrose, 100 µg per ml gentamycin, and 500 µg per ml carbenicillin.

**The generation of antibodies to CmSAMS**

The full-length CmSAMS coding region (393 aa) was cloned into the Escherichia coli expression vector pQE80L (Qiagen, Chatsworth, CA) to produce the His-tagged recombinant proteins. These recombinant proteins were purified with a HisTrap kit (Amersham Pharmacia), and the resulting CmSAMS protein was used to immunize guinea pigs to produce the anti-CmSAMS antisera.

**Genomic PCR, RT-PCR and immunoblotting of transgenic A. thaliana**

Genomic PCR, RT-PCR and immunoblotting were carried out using genomic DNA, RNA and soluble protein extracted from mature leaves, respectively. The RNeasy Plant Mini Kit (QIAGEN) was used to extract RNA. cDNA was synthesized with 0.5 µg of RNA using SuperScript® VILO™ cDNA synthesis kit (Invitrogen) and Ribonuclease Inhibitor (Super) (Wako). Soluble protein was extracted from mature leaves that were frozen in liquid nitrogen, ground with a mortar and pestle, and homogenized in extraction buffer (50 mM Tris–Mes, pH 7.5, 300 mM sucrose, 150 mM NaCl, 10 mM potassium acetate, 5 mM EDTA, and a protease inhibitor mixture [Complete EDTA-free; Roche Diagnostics]) with 0.5% Triton X-100. After centrifugation at 10,000×g for 10 min to exclude cell debris, the supernatant was mixed with an equal volume of 2X SDS gel loading buffer and used as the soluble protein fraction. PCR for confirmation of transgenic A. thaliana was performed using the following primers: Primer set 1 (cmSAM synthetase_F_692, 5’-AACGAGGTACGTGCTGACC-3’ and cmSAM synthetase_R_1152, 5’-CCAGGTTAAGTGGGATCATC-3’), Primer set 2 (AthSAM2CD_F, 5’-TGACAAAGCTTGTGGACCCA-3i and AthSAM2CD_R, 5’-TGAGGTCAGCCGCAATC-3’), and Primer set 3 (CMV35SPF2, 5’-CCTCTATATAAGGAAGCTCATTTCATTTGGA-GAGGACACG-3’ and AthSAM2CD_R2, 5’-CCACCTTCATGAGCTCCCCATCC-3’). GAPDH gene (glyceraldehyde-3-phosphate dehydrogenase) was amplified as positive control (AtGAPDH_F, 5’-TGAGGAGCACTTGGATAC-3’ and AtGAPDH_R, 5’-GGGAGAAGGGACGGTTTCG-3’).

**Estimation of high-salt tolerance of transgenic plants**

Wild-type and SAMS plants of A. thaliana were germinated and grown in 90-mm plastic Petri dishes on MS agar media containing 1% sucrose and 100 µg per ml carbenicillin at 0.1, 0.15, 0.175 M NaCl for 7 days. The A. thaliana plants were grown at 23°C in a growth chamber (LH-30-8CT; NKsystem, Osaka, Japan) equipped with white fluorescent lamps under a 12-h light/12-h dark photoperiod at a light intensity of 70 µmol m⁻² s⁻¹. For the salt tolerance assay, transgenic and wild-type seeds were planted on MS agar plates for germination. Seedlings from each line were transferred to a MS agar plate supplemented with different concentrations of NaCl. After 7 days of
Results and discussion

RT-PCR analysis of the genes of enzymes involved in the biosynthetic pathway of spermidine and spermine

Because the *A. thaliana* plants and *C. merolae* cells died at concentrations higher than 0.1 M and 0.4 M NaCl, respectively (Sakajiri *et al.* 2008), we used 0.3 M NaCl in the quantitative real-time RT-PCR experiments. RT-PCRs of 6 genes (ACC, ADC, SPDS, SPMS, SAMDC and
CmSAMS) that are involved in the pathway of spermidine and spermine were carried out. CmSAMS was remarkably highly expressed (4 to 5 expression ratio) after incubation for 2 h and 24 h in 0.3 M NaCl (Fig. 1A). SAMDC and ACC were also expressed at a higher level than was indicated from our microarray data. Although the RT-PCR experiments indicate higher expression levels for these genes, the results are nevertheless in accordance with our earlier microarray data (Sakajiri et al. 2008). On the other hand, SPDS, SPMS and ADC were expressed relatively at a lower level than was indicated from the microarray data. Therefore, it is suggested that the degradation of the transcripts of these genes is restrained. There are some reports that suggest that SAMDC and SPDS can confer tolerance to salt and drought stress in higher plants (Roy and Wu 2002, Waie and Rajam 2003, Kasukabe et al. 2004, 2006). However, because of its much higher

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**Fig. 2.** A phylogenetic tree based on SAM sequences (47 eukaryotes and 2 cyanobacteria) that was constructed using the maximum likelihood method. Bootstrap values are shown in the order NJ/MP/ML; values less than 50% are represented by “-”. GenBank accession numbers or JGI sequence ID number follow the species names. The tree is rooted at cyanobacteria.
expression level, we opted to examine the ability of the CmSAMS gene to produce stress tolerance to salt and drought in *A. thaliana*.

**Characterization and phylogenetic position of CmSAMS**

From the open reading frame of the CmSAMS gene, the product was identified as a 393 aa, 43.41 kDa protein. When CmSAMS isoforms from various species (*A. Arabidopsis thaliana, Oryza sativa, Chlamydomonas reinhardtii, C. merolae, Aureococcus anophagefferens*) were compared, the full-length CmSAMS peptide was shown to have a number of conserved regions (3 large conserved domains at the N-terminal and a semi-conserved domain at the C-terminal) (Fig. 1B, C). The phylogenetic tree for CmSAMS and the previously known eukaryotic SAMS was constructed (Fig. 2). CmSAMS formed a clade with the SAM of *A. anophagefferens*, a marine picoplankton that belongs to the class Pelagophyceae (division Heterokontophyta). While the SAM of *A. anophagefferens* showed strong affinity to CmSAMS (96/76/94), 2 other heterokontophytes, centric diatom *Thalassiosira pseudonana* and parasitic *Phytophthora infestans*, formed a robust clade (bootstrap 95/92/96). This topology suggests that the SAMS of *C. merolae* and *A. anophagefferens* share some common features relate to halotolerance. In addition, the semi-conserved domains of SAMS in both these algae differ from those found in freshwater green alga or non-halophytic land plants (Fig. 1B). These results may reflect the fact that both *C. merolae* and *A. anophagefferens* inhabit high ionic environments. Further analysis using a more comprehensive dataset of SAMS
protein sequences from a more diverse eukaryotic groups, especially including other rhodophytes, could help resolve the phylogenetic position and significance of CmSAMS.

Expression and localization of the CmSAMS-GFP fusion protein in the plant cells

To examine the location of CmSAMS proteins in onion cells, we constructed a chimeric gene by fusing the full-length CmSAMS gene and the GFP gene under the control of the CaMV35S promoter (Fig. 3A). GFP-vector, mitochondria (mt)-targeted GFP and plastid (pt)-targeted GFP genes were also used as markers (Fig. 3A). These constructs were introduced in onion epidermis cells using the particle-bombardment method. As predicted, the fluorescence signals from the GFP proteins, mt-GFP and pt-GFP were located in the cytoplasm, mitochondria and plastid, respectively. As for the mt- or pt- targeted GFP, the fluorescence was also seen in the outskirts of the cell.
nucleus. Since it is known that the sGFP accumulated diffusely in the cytoplasm and cell nucleus without a targeting sequence (Chiu et al. 1996), there may be some GFP molecules without the correct signal peptide. The signal from the CmSAMS-GFP fusion protein was located in the cytoplasm and cell nuclei in onion cells (Fig. 3B), indicating that CmSAMS was localized there.

Construct of CmSAMS to produce transgenic A. thaliana for NaCl tolerance

To obtain transgenic plants for NaCl tolerance, we prepared constructs containing the full-length coding region of CmSAMS and AtSAMS fused to the CaMV35S promoter (Fig. 4A). These

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**Fig. 5.** The seeds from wild-type and transgenic lines (CmSAMS) 1–4 were cultured in MS medium containing 0.15 M NaCl (A, B). Wild-type plants (WT) elongated slightly but the growth of the white-yellow cotyledons ceased. By contrast, the CmSAMS 1 and 4 cotyledons retained their green color although some of the cotyledons showed minor bleaching (A, B). Wild-type and CmSAMS 1 were incubated in 0.175 M NaCl and their cotyledons were examined in squashed preparations for fluorescence microscopy. Wild-type strains showed remarkably bleaching, while CmSAMS 1 retained a pale green color (middle in A). Wild-type cotyledon emitted weak red autofluorescence, while CmSAMS 1 emitted strong (bottom in A).
constructs were introduced into *A. thaliana* by the dipping method with *Agrobacterium*. To confirm transgenic plants by genomic PCR and RT-PCR, we designed 3 sets of primers (Fig. 4A). Primer set 1 and 2 were designed within the coding region of CmSAMS, and AtSAMS, respectively. Primer set 3 was designed to amplify the region including the portion of the CaMV35S promoter to avoid amplification of endogenous AtSAMS. As shown in Fig. 4, except for the wild-type plant, the introduced CmSAMS genes were confirmed in 4 transgenic lines (CmSAMS 1-4) by genomic PCR with primer set 1 (Fig. 4B, C left). Similarly, using Primer set 3, the introduced AtSAMS genes were confirmed in 2 transgenic lines (AtSAMS 1, 2, Fig. 4C right). The expression of the exogenous CmSAMS genes in the 2 representative lines 1 and 2 was confirmed by RT-PCR (Fig. 4D). Using extracted total RNA, cDNA was synthesized. PCR reaction was performed with total RNA and cDNA as templates. No signal was obtained when total RNA was used as template. This result suggested there was no contamination of genomic DNA in total RNA. Transcripts of CmSAMS were detected only in transgenic lines 1 and 2, while the endogenous transcripts of AtGAPDH (glyceraldehyde-3-phosphate dehydrogenase, the loading control) were amplified from all wild-type and transgenic plants (Fig. 4D). When RT-PCR was performed with AtSAMS plants, transcript of AtSAMS was increased compared with wild-type. We also performed immunoblot analyses of soluble proteins extracted from mature leaves of these plants using anti-CmSAMS antibody (Fig. 4E). The anti-CmSAMS antibody detected AtSAMS in wild-type plants probably because of the high homology between AtSAMS and CmSAMS. CmSAMS plants showed overexpression of CmSAMS protein, however, in spite of the expression of RNA (Fig. 4D), the expression of AtSAMS in transgenic AtSAMS plants was dramatically decreased (Fig. 4E). When the Atsaml gene was overexpressed in tobacco, the transgenic plant exhibited various expression patterns for Atsaml and the expression was translationally and/or post-translationally regulated (Boerjan *et al.* 1994). Similarly, the overexpression of AtSAMS might affect endogenous AtSAMS translation in AtSAMS plants.

**Estimation of salt tolerance of transgenic *A. thaliana* plants**

To examine the possible phenotypes of transgenic lines, T3 progeny of the CmSAMS-overexpressed lines and of the wild-type plants were grown in the growth chamber. To test the effect of CmSAMS overexpression on salt tolerance, the seeds from wild-type and CmSAMS transgenic lines 1–4 were cultured in MS medium containing 0.1 M and 0.15 M NaCl (Figs. 4B and 5A, B). No difference was observed in the phenotypes of the transformants and of the wild-type when the seeds were incubated in 0.1 M NaCl (data not shown). However, wild-type plants grown in 0.15 M NaCl displayed severe reduction in size and the beginnings of leaf chlorosis (Fig. 5A, B).

CmSAMS transgenic line 1 was grown on MS agar plates containing 0.175 M NaCl. The transgenic plant lines except lines 3 and looked normal and still grew after treatment with up to 0.15 M NaCl. Notably, the juvenile seedlings of one of the 4 CmSAMS transgenic lines (line 4) displayed strong salt tolerance (Fig. 5B). CmSAMS transgenic line 1 showing middle tolerance was examined in detail. When the wild-type and CmSAMS plants were germinated on MS agar media containing 0.175 M NaCl, the chlorophyll content in wild-type decreased markedly from the cotyledons while in CmSAMS, chlorophyll remained slightly (Fig. 5A). To quantify this, we examined the autofluorescence emitted from the chlorophyll of both the wild-type and the transgenic plants using the VIMPCS system. The autofluorescence from CmSAMS cotyledons was 20 times higher than from the cotyledons of the wild-type (Table 1). Since chlorophyll autofluorescence quantified by VIMPCS analysis is proportional to the chlorophyll content determined spectrophotometrically (Hirooka *et al.* 2009), the chlorophyll content in wild-type leaves decreased

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<th>Concentration</th>
<th>Fluorescent intensity (Cm/WT)</th>
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<tr>
<td>0.15 M</td>
<td>2.7±0.6</td>
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<tr>
<td>0.175 M</td>
<td>20.1±11.3</td>
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Table 1. Chlorophyll content
markedly from the content in the cotyledons, while in CmSAMS leaves and cotyledons it remained.

Under the NaCl stress conditions, the salt tolerance of the AtSAMS-overexpressed transgenic plants was weak as compared with CmSAMS plant (data not shown). Our results clearly indicate that the introduction of the CmSAMS gene confers tolerance to salt stress in *A. thaliana*.

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