Overexpression of *S-Adenosylmethionine Synthetase* Gene from *Pyropia tenera* Enhances Tolerance to Abiotic Stress

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ABSTRACT *Pyropia tenera* is an intertidal red alga of commercial significance owing to its popularity as a health-promoting seafood product. This alga grows in marine environments and is frequently exposed to high salinity and osmotic stress, which impact its growth. Therefore, the enhancement of stress tolerance in *P. tenera* is critical. In the present work, we aimed to elucidate the mechanisms underlying abiotic stress tolerance in this species; specifically, we identified the *P. tenera* S-adenosylmethionine synthetase-encoding gene (*PtSAMS*) and characterized its biological function. This gene, which is known to play a role in stress tolerance in other plants, was cloned and overexpressed in *Escherichia coli* under high-salinity conditions. The *PtSAMS* gene was found to encode a 385-amino-acid protein with a molecular weight of 41.8 kDa. In silico sequence alignment and phylogenetic analysis of the *PtSAMS* amino acid sequence showed that the encoded protein comprises three conserved domains and two motifs that are highly conserved in other plants. Growth assay results indicated that *PtSAMS*-overexpressing *E. coli* cells exhibit enhanced tolerance to salt stress. The results suggest that *PtSAMS* expression is induced by a combination of ion toxicity and osmotic stress resulting from exposure to high salinity in marine environments, and that this gene is expressed at housekeeping levels owing to growth in such conditions. The findings suggest that *PtSAMS* could be used as a potentially valuable bioresource with utility in the genetic engineering of salt stress-tolerant crop plants.

Keywords S-adenosylmethionine synthetase (SAMS), *Pyropia tenera*, Abiotic stress, Salinity, Recombinant protein, Tolerance

INTRODUCTION

Algae have been consumed as edible seaweed in Asia for thousands of years. *Pyropia* (Rhodophyta) have been cultivated since the 17th century in Korea, Japan, and China and represent commercially valuable seafood. *Pyropia*, which is highly popular owing to its delicious taste, contains high levels of protein and is rich in essential amino acids and vitamins (Blouin *et al.* 2011). The market for *Pyropia* is worth about 7 trillion, and its exports have experienced recent growth. It is expected that the *Pyropia* market size would continue to increase as a result of its recognition as a health-beneficial food product.

The environment in which seaweed is cultivated is exposed to harsh marine conditions. Aquaculture produc-

tivity is affected by temperature, rainfall, liver disease, and other factors. Therefore, the identification of rapidly growing or disease-resistant seaweed species is critical.

Salinity is one of the major abiotic factors that limit crop growth and productivity. Salt stress results in excessive ion toxicity, oxidative stress, and physiological drought, consequently causing disruption of cell organelles and their metabolism, eliciting nutrient imbalance in the plant, and reducing its osmotic potential (Tester and Bacic 2005). These effects lead to arrest of growth and development of the plant, thereby limiting its survival.

S-Adenosylmethionine synthetase (SAMS) catalyzes the conversion of adenosine 5'-triphosphate (ATP) and methionine into S-adenosylmethionine (SAM) (Pajares and Markham 2011). SAM plays a central role in diverse

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biological processes, such as by serving as a methyl donor during transmethylation in plants, and acting as a common precursor in the biosynthesis of polyamines and ethylene (Kumar *et al.* 1997; Roeder *et al.* 2009). The SAMS-encoding gene has been cloned from various species such as bacteria, yeasts, humans, animals, and plants. In plants, SAMS has been reported to play a role in developmental process and the stress response (Pulla *et al.* 2009). In *Arabidopsis thaliana*, the *SAMS* gene plays a role in seed germination, as shown in a previous study of MTO3 mutants that showed delayed germination and high level concentration of methionine phenotype. Knockdown of the *SAMS* gene resulted in delayed flowering time and dwarf phenotype in rice (Boerjan *et al.* 1994; Li *et al.* 2011). Expression of *SAMS* is induced under low temperature in *Arabidopsis*, rice, and maize (Cui *et al.* 2005; Amme *et al.* 2006; Yang *et al.* 2006; Uvackova *et al.* 2012). *Pisum sativum* *SAMS1* is expressed during pea development (Gómez-Gómez and Carrasco 1998). Overexpression of the stress-inducible gene *GsSAMS2* enhances salt tolerance in transgenic *Medicago sativa* (Hua *et al.* 2012). *Suaeda salsa* inhabits saline or alkaline soil such as coastal salt-flats. Recently, overexpression of *S. salsa* *SAMS* was shown to increase salt tolerance in transgenic tobacco (Qi *et al.* 2010). Although *Porphyra yezoensis* *SAMS* (*PySAMS*) has been cloned (Yi *et al.* 2009), its function is still unknown.

Here, we reported the cloning and characterization of the *SAMS* (*PtSAMS*) gene in *Pyropia tenera*. In order to elucidate the function of *PtSAMS*, we additionally examined the expression of *PtSAMS* in *E. coli* under stress conditions. Functional analysis in *E. coli* suggested that *PtSAMS* plays a role in the tolerance to stress.

MATERIALS AND METHODS

Plant materials

The *P. tenera* strain used in this study was received from the Seaweed Research Center, National Research & Development Institute. *P. tenera* was cultured at 10°C in a growth chamber with bubbling under 50 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ and a photoperiod of 12 hour light and 12 hour dark. The culture

media used was Provasoli's enrichment solution (PES); the media were changed every week (Kakinuma *et al.* 2016).

RNA extraction, reverse transcription, and isolation of *PtSAMS* gene

The *PtSAMS* sequence was obtained by performing a search of the NCBI (National Center of Biotechnology Information) database, based on the sequence of *Porphyra yezoensis* *S-adenosylmethionine synthetase* (*SAMS*) gene. Total RNA was extracted from *P. tenera* with Hybrid-R (GeneAll, Seoul, Korea) according to the manufacturer's guide. The first-strand cDNA was synthesized using amfiRivert cDNA Synthesis Platinum Master Mix (GenDEPOT, Texas, US). The full-length *PtSAMS* gene was isolated through RT-PCR using the *PtSAMS*-F (5'-CACCATGGCAGCCATGAAG-3') and *PtSAMS*-R (5'-ACGACGCTCTAGAGCTCAC-3') primers based on the *P. yezoensis* sequence (GenBank accession: FJ404748) (Table 1).

Cloning of *PtSAMS* gene into the pET28(b) expression vector and recombinant protein expression

PtSAMS was amplified by PCR using the *PtSAMS*-*Nde*I-F (5'-CCCCATATGGCAGCCATGAAGA-3') and *PtSAMS*-*Xho*I-R (5'-TCACTCGAGGAGCTCAAGC-3') primers with flanking restriction sites of *Nde*I and *Xho*I, respectively (Table 1). The PCR product was digested with *Nde*I and *Xho*I restriction endonucleases and cloned into pET28(b). The pET28(b)-*PtSAMS* plasmid was transformed into the *E. coli* strain BL21(DE3). The pET28(b) empty vector was used as a control in spot assay and liquid growth assay of stress treatment (Yadav *et al.* 2012).

Sequence analysis

PtSAMS and protein sequences of other *SAMS* of different species were searched in the NCBI database. Amino acid alignments were conducted using the ClustalW algorithm implemented in BioEdit (Thompson *et al.* 1994). The phylogenetic tree of the *SAMS* protein was constructed using the Geneious software 9.0.4. using a neighbor-joining (NJ) method with bootstrap set at 10,000 replicates.

Table 1. Gene specific primers used in the study.

Name	Sequence (5'-3')	Purpose
<i>PtSAMS</i> -F	CACCATGGCAGCCATGAAG	For full length of <i>PtSAMS</i> gene
<i>PtSAMS</i> -R	ACGACGCTCTAGAGCTCAC	For full length of <i>PtSAMS</i> gene
<i>PtSAMS</i> -NdeI-F	CCCC ATAT GGCAGCCATGAAGA ²⁾	For protein expression
<i>PtSAMS</i> -XhoI-R	TCA CTCGAGG AGCTCAAGC ²⁾	For protein expression
<i>PtSAMS</i> -303-F	CCAGTCCCCTGAGATTGCTG	Probe for southern blot analysis
<i>PtSAMS</i> -955-R	CTGAAATCGGCTCGGCAATG	Probe for southern blot analysis
<i>PtSAMS</i> -406-F	GAGGACCTGATGCCCTTGAC	RT-qPCR
<i>PtSAMS</i> -591-R	CTGCGTGCTGATGACAATCG	RT-qPCR
<i>PtGAPDH</i> -2771-F	CGCCGAGTACATTGTCGAGT	RT-qPCR
<i>PtGAPDH</i> -3002-R	GTA CTTCT CGTGCAGCACCT	RT-qPCR

²⁾Bold letters indicate restriction enzyme sites.

Southern blot analysis

Genomic DNA was isolated from *P. tenera* using the DNeasy Plant Maxi-prep Kit (Qiagen, Hilden, Germany). Genomic DNA (5 µg) was digested with *Bam*HI and *Kpn*I, separated on 0.7% agarose gel, and transferred to Hybond-N+ membrane. The membrane was treated by UV crosslinking to fix the genomic DNA. Probe DNA was prepared by PCR for a specific region of *PtSAMS*. The gene-specific primers used were *PtSAMS*-303-F (5'-CCAGTCCCCTGAGATTGCTG-3') and *PtSAMS*-955-R (5'-CTGAAATCGGCTCGGCAATG-3') (Table 1). Probe labeling with dioxigenin (DIG), hybridization, and detection were carried out using the DIG High Prime DNA Labeling and Detection Starter Kit I (Roche, Basel, Switzerland) according to the manufacturer's instructions.

Quantitative RT-PCR analysis

cDNA from *P. tenera* was used as a template for qRT-PCR using *PtSAMS* gene-specific primers *PtSAMS*-303-F and *PtSAMS*-955-R. Real time qPCR was performed using the Takara PCR system with a SYBER GREEN KIT (Takara, Shiga, Japan). PCR conditions were as follows: 94°C for 2 minutes, 40 cycles at 94°C for 30 seconds, 62°C for 30 seconds, 72°C for 30 seconds, and a final extension at 72°C for 10 minutes. The experiments were repeated three times independently. The *PtGAPDH* gene was used as an internal control under the same conditions. Primer sequences were as follows: *PtGAPDH*-2771-F (5'-CGCCGAGTACATTGTCGAGT-3') and *PtGAPDH*-3002-R

(5'-GTACTTCTCGTGCAGCACCT-3') (Lee *et al.* 2015) (Table 1).

Spot culture assay for analysis of the effects of stress treatment

pET28(b)-*PtSAMS*/BL21(DE3) cells were grown in LB medium to OD₆₀₀ = 0.6, and 0.1 mM IPTG was added (Yadav *et al.* 2012). The induced cells were grown for 5 hours at 37°C. The cultured cells were diluted to an OD₆₀₀ of 0.6, and then to 10⁻³, 10⁻⁴, and 10⁻⁵. Ten micro liters from each dilution was spotted on to an LB-only plate, or an LB plate supplemented with 400 mM NaCl and 400 mM KCl. The experiments were repeated three times independently.

Liquid culture assay under stress treatment

Growth rate analysis was tested in LB liquid medium or in LB medium supplemented with NaCl and KCl. Then, 400 µL of pET28(b)-*PtSAMS* recombinant or vector alone in *E. coli* BL21 cells were inoculated into 10 mL of LB liquid medium for overnight culture. The next day, the cultured cells were diluted to an OD₆₀₀ of 0.6 and 0.1 mM IPTG was added for induction. Cells were grown for 5 hours at 37°C and then were diluted to OD₆₀₀ of 0.6. Next, 500 µL of cells were inoculated into 50 mL of LB medium containing 400 mM NaCl, and 400 mM KCl, or LB medium only, and cultured at 37°C. Cells were harvested every 1 hour, until 12 hours, and OD₆₀₀ values were measured.

RESULTS

Isolation and characterization of *PtSAMS*

To isolate the *PtSAMS*, we designed primer sets based on the *P. yezoensis* sequence (Accession number: FJ404748) in the NCBI GenBank sequence database (Table 1). The *PtSAMS* gene was cloned from the cDNA library by PCR and sequenced (data not shown). Sequence analysis showed that the complete open reading frame of *PtSAMS* is 1,155 bp in length, and is composed of one exon. The predicted *PtSAMS* protein comprises 385 amino acid residues with a calculated molecular weight of 41.8 kDa and isoelectric point (PI) of 5.60.

Sequence analysis and phylogenetic analysis of *PtSAMS*

To further characterize the *PtSAMS* protein, we performed a conserved domain (CD) analysis through the GenomeNet Database Resources (<http://www.genome.jp/tools/motif/>). The predicted *PtSAMS* protein consisted of three S-adenosylmethionine synthetase N-terminal, central, and C-terminal domains. Furthermore, the predicted *PtSAMS* sequence contained two substrate-binding sites, as well as a site for ATP binding in the central domain and for Met binding in the C-terminal domain (Fig. 1a). The BioEdit software was used for comparison of the amino acid sequence of *PtSAMS* with SAMS from other representative Rhodophyta, Phaeophyta, and Chlorophyta (Fig. 1a). The results showed that *PtSAMS* shared higher homology with SAMS from plant species such as *Galdieria sulphuraria* (76%), *Ectocarpus siliculosus* (70%), *Chlamydomonas reinhardtii* (69%), and *A. thaliana* (66%). To investigate the evolutionary relationship of *PtSAMS* protein among SAMS proteins of other species, a phylogenetic tree was constructed using Geneious 9.0.4 software on the basis of the multiple amino acid sequences (Fig. 1b). GenBank accession numbers of the protein sequences are shown in Table 2. Phylogenetic tree analysis revealed that the *PtSAMS* of Bangiophyceae was grouped with red algae, showing the highest degree of clustering with Rhodophyta, *Galdieria sulphuraria*, and then with SAMS of diatoms and Phaeophyta, followed by Chlorophyta. Table 3 shows pairwise comparison of the amino acid

sequences between *PtSAMS* and other species. The SAMS sequence was found to be highly conserved among all the different species.

Fig. 1. Amino acid sequence alignment of *PtSAMS* homologs and phylogenetic tree of SAMS from various species. (a) Comparison of amino acid sequences of *Pyropia tenera* SAMS with the SAMS of other species: *Galdieria sulphuraria* (XP_005703068.1), *Ectocarpus siliculosus* (CBJ30117.1), *Chlamydomonas reinhardtii* (XP_001696661.1), and *Arabidopsis thaliana* (NP_171751.1); identical residues are shaded black, and similar residues are shaded gray. The domains are indicated by underlining. Met-binding motifs are indicated by boxes. Dotted boxes indicate ATP-binding sites. (b) Phylogenetic tree analysis using Geneious software using Jukes Cantor, Neighbor Joining tree building method with a 10,000 bootstrap repeat value.

Table 2. GenBank and NCBI reference sequence accession numbers of SAMS sequences.

	Species	Amino acid sequence length	NCBI reference sequence
Rhodophyta	<i>Pyropia yezoensis</i>	384	ACJ98094.1
	<i>Galdieria sulphuraria</i>	393	XP_005703068.1
Phaeophyta	<i>Ectocarpus siliculosus</i>	397	CBJ30117.1
	<i>Undaria pinnatifida</i>	397	AEK80411.1
Chlorophyta	<i>Ostreococcus tauri</i>	386	XP_003083240.1
	<i>Chlamydomonas reinhardtii</i>	390	XP_001696661.1
	<i>Arabidopsis thaliana</i>	393	NP_171751.1
	<i>Oryza sativa</i>	396	AAT94053.1
Diatoms	<i>Thalassiosira pseudonana</i>	450	XP_002288884.1
	<i>Fistulifera solaris</i>	384	GAX17078.1
Bacterial	<i>Escherichia coli</i>	384	AAA24164.1

Table 3. Amino acid sequence identity matrix comparison of PtSMAS with SAMS of other species.

	PtSAMS	GsSAMS	CrSAMS	EsSAMS	AtSAMS
PtSAMS	100%	76.84%	70%	69.6%	66.75%
GsSAMS		100%	69.77%	68.91%	67.59%
CrSAMS			100%	65.92%	81.14%
EsSAMS				100%	64.25%
AtSAMS					100%

Southern blot and expression analysis of *PtSAMS* at different phases of the life cycle

To determine the copy number of *SAMS* in the *P. tenera* genome, genomic DNA of *P. tenera* was digested with restriction endonucleases *Bam*HI and *Kpn*I; then, Southern blot analysis was performed. After transferring to a membrane, the separated genomic DNA was hybridized to DIG-labeled *PtSAMS* gene-specific probe. Southern blot analysis result clearly showed that the *PtSAMS* gene was present as a single copy in *P. tenera* (Fig. 2). To examine the expression levels of *PtSAMS* transcripts at different life cycles, qPCR analysis was performed (Fig. 3). Total RNA was isolated from the conchocelis and thallus of *P. tenera*. The relative mRNA expression level in the thallus was higher than that in conchocelis by approximately three folds.

Expression analysis of recombinant PtSAMS protein in *E. coli* by SDS-PAGE

The complete ORF of the *PtSAMS* gene was cloned into a pET28(b) vector and expressed in the *E. coli* strain

Fig. 2. Southern blot analysis of *P. tenera* genomic DNA: 5 µg of genomic DNA per lane was digested with restriction enzymes and separated by electrophoresis in a 0.7% agarose gel. The DNA was transferred to a nylon membrane and probed with a DIG-labeled *PtSAMS* cDNA fragment. Molecular size markers are indicated on the left.

BL21(DE3). The empty pET28(b) vector was used as a control in this experiment. Expression of the recombinant PtSAMS protein was induced by adding 0.1 mM IPTG after 1 hour; this reached a maximum at 7 hours (Fig. 4a).

To confirm the production of His tag-fused PtSAMS, western blot analysis was carried out using anti-His (Fig. 4b). The theoretical molecular weight of the recombinant PtSAMS was about 44.0 kDa.

Growth of PtSAMS-expressing *E. coli* under abiotic stress

To examine the effect of the overexpression of the PtSAMS protein in *E. coli* under various abiotic stresses, the recombinant cells were spotted on LB medium supplemented with NaCl and KCl (Fig. 5). The pET28(b)-

PtSAMS and pET28(b) cells showed similar growth on LB medium in overnight grown culture (Fig. 5a). The pET28(b)-PtSAMS recombinant cells showed increased number of colonies in NaCl and KCl treatment compared with that in control cells (Fig. 5b and 5c). In addition, the growth rate of recombinant cells was analyzed in LB liquid medium. pET28(b)-PtSAMS recombinant and pET28(b) empty vector control BL21(DE3) cells were inoculated into fresh LB liquid medium and medium supplemented with NaCl and KCl. In LB liquid medium, pET28(b)-PtSAMS recombinant cell and cells harboring the pET28(b)

Fig. 3. Expression pattern analysis of PtSAMS at different life cycle stages in *P. tenera*: Total RNA was isolated from conchocelis and thallus of *P. tenera* at various life stages. The mRNA expression level of the conchocelis stage was set to 1. *PtGAPDH* was used as normalization control. The results are presented as average values with SD using each three times.

Fig. 5. Spot assay of pET28(b) and pET28(b)-PtSAMS recombinant protein in *E. coli* cells with NaCl and KCl treatment; 10 μ L from 10^{-3} to 10^{-5} dilution series were spotted on (a) control (LB-only) plate, or (b) LB plate supplemented with 400 mM NaCl, and (c) 400 mM KCl.

Fig. 4. SDS-PAGE and western blot analysis of PtSAMS recombinant protein in *E. coli* BL21(DE3). (a) SDS-PAGE analysis. M: marker, Lane 1: uninduced protein, Lane 2-4: induced protein by 0.1 mM IPTG for 1, 3, 5 (hour), respectively. (b) Analysis of expression of recombinant PtSAMS protein in *E. coli* BL21(DE3) cells; western blotting analysis of the PtSAMS recombinant proteins with anti-His tag antibodies. Arrows indicate the His-tagged PtSAMS recombinant protein.

vector alone showed similar growth at various times. In the NaCl- and KCl-treatment treatment, pET28(b)-*PtSAMS* recombinant cells showed better growth compared with cells harboring the vector alone 6 hours after inoculation (Fig. 6). Therefore, the spot and liquid culture assay indicated a similar pattern between the groups in terms of the abiotic stress response.

Fig. 6. Growth analysis of pET28(b) and pET28(b)-*PtSAMS* recombinant protein in *E. coli* cells on liquid medium with NaCl and KCl treatment; (a) LB medium, (b) 400 mM NaCl, and (c) 400 mM KCl. OD₆₀₀ was recorded at 1 hour interval up to 12 hours and mean values are represented in graph.

DISCUSSION

Pyropia grows in high intertidal zones where this plant experiences environmental conditions such as desiccation, salinity fluctuations, intense radiation, and high temperatures; additionally, *Pyropia* is regularly submerged in seawater and exposed to air during high and low tides (Xu *et al.* 2017). This plant has developed mechanisms to resist salt stress and survive under severe conditions. However, the molecular mechanisms underlying the salt stress tolerance in Rhodophyta are not fully understood. In this study, we attempted to isolate the salt stress gene in seaweed by cloning the *S-Adenosyl-L-methionine synthetase* (*SAMS*) gene from *P. tenera*; this gene was referred to as *PtSAMS*. S-Adenosyl-L-methionine synthase synthesizes S-adenosyl-L-methionine (AdoMet) from L-methionine and ATP in both prokaryotes and eukaryotes (Boerjan *et al.* 1994). In silico sequence alignment and phylogenetic analysis of amino acid sequence showed that *PtSAMS* comprises three conserved domains: the N-terminal domain, central domain, and C-terminal domain (Fig. 1a). Furthermore, *PtSAMS* contains two motifs, Met-binding and ATP-binding sites, that are highly conserved in other plants. In the phylogenetic tree based on amino acid sequences of different *SAMS* proteins, *PtSAMS* was clustered with the *SAMS* from *G. sulphuraria*, Rhodophyta (Fig. 1b). Taken together, these results suggest that *PtSAMS* from *P. tenera* is a member of the red algae *SAMS* family. Further, we identified the presence of a single copy of the *SAMS* gene in the *Pyropia* genome by DNA gel blot analysis (Fig. 2). To examine *PtSAMS* expression at various stages of the life cycle, RT-qPCR amplification of DNA from the conchocelis and thallus of *P. tenera* was performed (Fig. 3). The relative mRNA expression level of *PtSAMS* in the thallus was higher than in the conchocelis. However, the expression level did not differ under exposure to abiotic stresses in the thallus (data not shown). As *Pyropia* inhabits sea water, this organism is exposed to environmental stresses such as high salt levels and drought, and is therefore adapted for growth and survival in the marine environment (Xu *et al.* 2017). It is therefore possible that the expression of the *PtSAMS* gene is maintained at the level of a housekeeping gene owing to

the constant exposure to such growing conditions.

Salinity stress is an important environmental factor that limits plant growth and development. SAM acts as a universal methyl group donor in biological processes during numerous specific trimethylations of protein, lipids, polysaccharides, and nucleic acids (Li *et al.* 2011). SAM additionally serves as precursor for polyamine biosynthesis (Evans and Malmberg 1989). The plant hormone, ethylene, is synthesized from SAM and participates in various physiological processes such as the stress response (Wang *et al.* 2002; Wang *et al.* 2013). *SAMS* is expressed in various tissues in other plant species (Li *et al.* 2011): in rice, the *SAMS* gene regulates ethylene-mediated inhibition of root development and alteration of cell wall structures (Fukuda *et al.* 2007). *SAMS* expression is associated with the mechanism underlying tolerance of plants to abiotic stresses; it has been shown that transgenic *SAMS*-expressing plants show enhanced resistance to abiotic stresses. In tomato, *SAMS* were differentially expressed after application with salt stress (Espartero *et al.* 1994). Overexpression of *SAMS* in *M. sativa* resulted in enhanced tolerance to cold stress by accelerating polyamine oxidation (Guo *et al.* 2014). In addition, the levels of three *SAMS* transcripts were increased transiently following the application of various stresses in *Catharanthus roseus*; in particular, a high degree of accumulation of *SAM2* transcripts was observed (Schroder *et al.* 1997). Taken together with the results of previous studies, the present findings suggest that the expression of the *SAMS* gene maybe induced by a combination of ion toxicity and osmotic stress during exposure to salt stress.

E. coli growth assay results showed that pET8(b)-*PtSAMS* recombinant *E. coli* cells showed better tolerance to salt stress than cells harboring the control vector (Fig. 5). Similar to our results, previous studies have reported that the expression of stress-induced genes enhances tolerance to various stresses in other plants. Overexpression of *Lycoris radiata* *SAMS* recombinant protein was induced effectively under high salinity (NaCl and KCl) compared with that in control samples (Li *et al.* 2013; Cui *et al.* 2005). Ma *et al.* (2003) found that the expression of the *SAMS* gene from *Suaeda salsa* is induced under NaCl stress (Ma *et al.* 2003). Recently, it has been reported that the *AvSAMS*

gene confers aluminum stress tolerance and facilitates epigenetic gene regulation (Ezaki *et al.* 2016). *SAMS* is therefore known to play roles in various plant defense systems. The present results indicate that *PtSAMS* maybe plays an important role in salt tolerance. This gene therefore suggests a potential bioresource for the genetic engineering of abiotic stress tolerance in plants.

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REFERENCES

- Amme S, Matros A, Schlesier B, Mock HP. 2006. Proteome analysis of cold stress response in *Arabidopsis thaliana* using DIGE-technology. *J. Exp. Bot.* 57: 1537-1546.
- Blouin NA, Brodie JA, Grossman AC, Xu P, Brawley SH. 2011. *Porphyra*: a marine crop shaped by stress. *Trends Plant Sci.* 16: 29-37.
- Boerjan W, Bauw G, Van Montagu M, Inze D. 1994. Distinct phenotypes generated by overexpression and suppression of S-adenosyl-L-methionine synthetase reveal developmental patterns of gene silencing in tobacco. *Plant Cell* 6: 1401-1414.
- Cui S, Huang F, Wang J, Ma X, Cheng Y, Liu J. 2005. A proteomic analysis of cold stress responses in rice seedlings. *Proteomics* 5: 3162-3172.
- Espartero J, Pintor-Toro JA, Pardo JM. 1994. Differential accumulation of S-adenosylmethionine synthetase transcripts in response to salt stress. *Plant Mol. Biol.* 25: 217-227.
- Evans JM, Malmberg RL. 1989. Do polyamines have roles in plant development? *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 40: 235-269.
- Ezaki B, Higashi A, Nanba N, Nishiuchi T. 2016. An S-adenosyl methionine synthetase (*SAMS*) gene from *Andropogon virginicus* L. confers aluminum stress tolerance and facilitates epigenetic gene regulation in *Arabidopsis thaliana*. *Front. Plant Sci.* 7: 1627.

- Fukuda T, Saito A, Wasaki J, Shinano T, Osaki M. 2007. Metabolic alterations proposed by proteome in rice roots grown under low P and high Al concentration under low pH. *Plant Sci.* 172: 1157-1165.
- Gómez-Gómez L, Carrasco P. 1998. Differential expression of the S-adenosyl-L-methionine synthase genes during pea development. *Plant Physiol.* 117: 397-405.
- Guo Z, Tan J, Zhuo C, Wang C, Xiang B, Wang Z. 2014. Abscisic acid, H₂O₂ and nitric oxide interactions mediated cold-induced S-adenosylmethionine synthetase in *Medicago sativa* subsp. *falcata* that confers cold tolerance through up-regulating polyamine oxidation. *Plant Biotechnol. J.* 12: 601-612.
- Hua Y, Zhang BX, Cai H, Li Y, Bai X, Ji W, Wang ZY, Zhu YM. 2012. Stress-inducible expression of GsSAMS2 enhances salt tolerance in transgenic *Medicago sativa*. *African Journal of Biotechnology* 11: 4030-4038.
- Kakinuma M, Nakamoto C, Kishi K, Coury DA, Amano H. 2017. Isolation and functional characterization of an ammonium transporter gene, *PyAMT1*, related to nitrogen assimilation in the marine macroalga *Pyropia yezoensis* (Rhodophyta). *Mar. Environ. Res.* 128: 76-87.
- Kumar A, Taylor M, Altabella T, Tiburcio AF. 1997. Recent advances in polyamine research. *Trends Plant Sci.* 2: 124-130.
- Lee MK, Kim IH, Choi YH, Choi JW, Kim YM, Nam TJ. 2015. The proliferative effects of *Pyropia yezoensis* peptide on IEC-6 cells are mediated through the epidermal growth factor receptor signaling pathway. *Int. J. Mol. Med.* 35: 909-914.
- Li W, Han Y, Tao F, Chong K. 2011. Knockdown of SAMS genes encoding S-adenosyl-L-methionine synthetases causes methylation alterations of DNAs and histones and leads to late flowering in rice. *J. Plant Physiol.* 168: 1837-1843.
- Li XD, Xia B, Wang R, Xu S, Jiang YM, Yu FB, Peng F. 2013. Molecular cloning and characterization of S-adenosylmethionine synthetase gene from *Lycoris radiata*. *Mol. Biol. Rep.* 40: 1255-1263.
- Ma X, Wang Z, Qi Y, Zhao Y, Zhang H. 2003. Isolation of s-adenosylmethionine synthetase gene from *Suaeda salsa* and its differential expression under NaCl stress. *Acta Botanica Sinica* 45: 1359-1365.
- Pajares MA, Markham GD. 2011. Methionine adenosyl-transferase (s-adenosylmethionine synthetase). *Adv. Enzymol. Relat. Areas Mol. Biol.* 78: 449-521.
- Pulla RK, Kim YJ, Parvin S, Shim JS, Lee JH, Kim YJ, In JG, Senthil KS, Yang DC. 2009. Isolation of S-adenosyl-L-methionine synthetase gene from *Panax ginseng* C.A. meyer and analysis of its response to abiotic stresses. *Physiol. Mol. Biol. Plants* 15: 267-275.
- Qi YC, Wang FF, Zhang H, Liu WQ. 2010. Overexpression of *suadea salsa* S-adenosylmethionine synthetase gene promotes salt tolerance in transgenic tobacco. *Acta Physiologiae Plantarum* 32: 263-269.
- Roeder S, Dreschler K, Wirtz M, Cristescu SM, van Harren FJ, Hell R, Piechulla B. 2009. SAM levels, gene expression of SAM synthetase, methionine synthase and ACC oxidase, and ethylene emission from *N. suaveolens* flowers. *Plant Mol. Biol.* 70: 535-546.
- Schroder G, Eichel J, Breinig S, Schroder J. 1997. Three differentially expressed S-adenosylmethionine synthetases from *Catharanthus roseus*: molecular and functional characterization. *Plant Mol. Biol.* 33: 211-222.
- Tester M, Bacic A. 2005. Abiotic stress tolerance in grasses. From model plants to crop plants. *Plant Physiol.* 137: 791-793.
- Thompson JD, Higgins DG, Gibson TJ. 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res.* 22: 4673-4680.
- Uvackova L, Takac T, Boehm N, Obert B, Samaj J. 2012. Proteomic and biochemical analysis of maize anthers after cold pretreatment and induction of androgenesis reveals an important role of anti-oxidative enzymes. *J. Proteomics* 75: 1886-1894.
- Wang F, Cui X, Sun Y, Dong CH. 2013. Ethylene signaling and regulation in plant growth and stress responses. *Plant Cell Rep.* 32: 1099-1109.
- Wang KL, Li H, Ecker JR. 2002. Ethylene biosynthesis and signaling networks. *Plant Cell* 14 Suppl: S131-151.
- Xu K, Xu Y, Ji D, Chen T, Chen C, Xie C. 2017. Cells tile a flat plane by controlling geometries during morphogenesis of *Pyropia thalli*. *PeerJ* 5: e3314.
- Yadav NS, Rashmi D, Singh D, Agarwal PK, Jha B. 2012. A novel salt-inducible gene *SbSI-1* from *Salicornia brachiata* confers salt and desiccation tolerance in *E. coli*. *Mol. Biol. Rep.* 39: 1943-1948.
- Yang PF, Li XJ, Liang Y, Jing YX, Shen SH, Kuang TY.

2006. Proteomic analysis of the response of Liangyoupeijiu (super high-yield hybrid rice) seedlings to cold stress. *J. Integr. Plant Biol.* 48: 945-951.

Yi LF, Wang P, Zhou X, Liu C. 2009. cDNA cloning and bioinformatic analysis of *SAMS* gene from *Porphyra yezoensis*. *China Biotechnology* 29: 43-49.