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Research article

Expression of potato S-adenosyl-L-methionine synthase (*SbSAMS*) gene altered developmental characteristics and stress responses in transgenic *Arabidopsis* plants

Sun Hee Kim [a,1,2], Sang Hyon Kim [a,2], Sasikumar Arunachalam Palaniyandi [b], Seung Hwan Yang [b,*], Joo-Won Suh [a,b,*]

[a] *Department of Bioscience and Bioinformatics, Graduate School, Myongji University, Yongin, Gyeonggi-Do 449-728, Republic of Korea*
[b] *Center for Nutraceutical and Pharmaceutical Materials, Myongji University, Cheoin-gu, Yongin, Gyeonggi-Do 449-728, Republic of Korea*

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ABSTRACT

S-adenosyl-L-methionine (SAM) synthase (SAMS) catalyze the biosynthesis of SAM, which is a precursor for ethylene and polyamines, and a methyl donor for a number of biomolecules. A full-length cDNA of SAMS from *Solanum brevidens* was expressed in *Arabidopsis thaliana* to study its physiological function. RT-PCR analysis showed that *SbSAMS* expression was enhanced significantly in *S. brevidens* leaves upon treatment with salt, mannitol, ethephon, IAA and ABA. The transgenic *SbSAMS* overexpression lines accumulated higher levels S-adenosyl homocysteine (SAHC) and ethylene concomitantly with increased SAM level. Expression levels of genes related to ethylene biosynthesis such as ACC synthase, but not polyamine biosynthesis genes were enhanced in *SbSAMS* overexpressing *Arabidopsis* lines. In addition, ABA responsive, wound and pathogen-inducible genes were upregulated in *SbSAMS* transgenic *Arabidopsis* plants. Transgenic *Arabidopsis* lines exhibited higher salt and drought stress tolerance compared to those of vector control. Based on these results we conclude that *SbSAMS* is expressed under abiotic stress to produce SAM as a broad-spectrum signal molecule to upregulate stress-related genes including ethylene and ABA biosynthetic pathway genes responsible for ABA, pathogen and wound responses.

1. Introduction

Plants are exposed to a variety of adverse conditions, such as cold, drought, and soils with ever-changing salt and nutrient concentrations (abiotic stresses) or herbivory and pathogen invasion (biotic stresses), which affect growth, development, and productivity. Plants have developed versatile strategies to cope with such stresses and their negative effects. In order to respond to stresses, plants make use of a multitude of physiological and biochemical ways resulting in induction of an array of functional or regulatory genes (Bartels and Sunkar, 2005).

S-adenosyl-L-methionine synthase gene (*SAMS*) is one of the functional genes induced during environmental stress. SAM synthase enzyme (SAMS) catalyze the biosynthesis of S-adenosyl-L-methionine (SAM), the universal methyl donor, from methionine and ATP. SAM possesses a highly reactive methylated sulphur required to methylate a range of biomolecules such as DNA, RNA, proteins, lipids, sterols, volatiles, pectin, lignin and flavonoids (Roeder et al., 2009; Gong et al., 2014). It also plays an important role in regulation of plant development, abiotic and biotic stresses (Gong et al., 2014). Beside the above functions, SAM is the intermediate in the biosynthesis of polyamines, nicotianamine, biotin and ethylene (Roeder et al., 2009).

A range of stresses trigger increase in ethylene biosynthesis in plants. In turn, ethylene plays a key role in response to the environmental cues. Precursor of ethylene is 1-aminocyclopropane-1-carboxylic acid (ACC), which is synthesized from SAM by ACC synthase (ACS). ACC is then converted to ethylene by ACC oxidase (ACO) (Wang et al., 2002). Alternatively, SAM is converted by SAM decarboxylase (SAMDC) to decarboxylated-SAM that donate the

* Corresponding authors. Center for Nutraceutical and Pharmaceutical Materials, Myongji University, Cheoin-gu, Yongin, Gyeonggi-Do 449-728, Republic of Korea.
E-mail addresses: ymichigan@mju.ac.kr (S.H. Yang), jwsuh@mju.ac.kr (J.-W. Suh).
[1] Present address: National Academy of Agricultural Science, Rural Development Administration, 370 Nongsaengnyeong-ro, Wansan-gu, Jeonju-si, Jeollabuk-do 560-500, Republic of Korea.
[2] These authors contributed equally to this work.

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aminopropyl group for polyamine biosynthesis. Polyamines also play an important role in plant tolerance to biotic and abiotic stress (Gong et al., 2014).

Induction of *SAMS* during stress results in increase in SAM level in plants that positively affects biosynthesis of both ethylene and polyamine sharing the molecule as a precursor (Roeder et al., 2009). Salt and cold stresses induced the *SAMS* transcript level in plants (Sanchez-Aguayo et al., 2004; Guo et al., 2014). *SAMS* expression is also regulated by a few plant hormones such as gibberellins (GA_3), α-naphthalene acetic acid (NAA), 6-benzylaminopurine (BAP), indole-3-butyric acid (IBA) and ABA (Guo et al., 2014).

These observations suggest that the stress response may be associated with increase in *SAMS*, eventually leading to production of ethylene, polyamine or other biomolecules. Increase in ethylene is associated with defense against attacks from pathogens or herbivores (biotic stress) (Arimura et al., 2002), while induced polyamine is correlated with defense against abiotic stress (Gong et al., 2014).

Molecular cloning and characterization of *SAMS* from a few plants species has been reported previously. Potato is an economically important crop belonging to the family *Solanaceae*. The productivity of potato around the world is threatened by biotic and abiotic stresses. In order to develop crops resistant to both abiotic and biotic stresses, engineering plants with genes that confer biotic and abiotic stress tolerance is essential. There was no report regarding *SAMS* from potato (*Solanum brevidens*) showing its physiological roles during stress. Recently, tomato plants (*Solanaceae* member) overexpressing SAM synthase gene was reported to show increased tolerance to alkali stress through polyamine metabolism (Gong et al., 2014). In this study, we analyzed the expression of *SbSAMS* in different potato organs and during various stress and hormone treatments. It has been previously reported that pest infestation and artificial wounding of lima bean leaves induced the expression of *SAMS*, *ACO*, and *SAMDC* genes (Arimura et al., 2002). To see whether *SbSAMS* overexpression in plants could lead to expression of genes responsive to wounding and pathogen attack, and to get insights into the physiological role of *SbSAMS*, we developed transgenic *Arabidopsis* plants expressing *SbSAMS* and observed responses and expression patterns of transgenic *Arabidopsis* lines upon stress and hormone treatments.

2. Materials and methods

2.1. Plant growth conditions

Wild type potatoes (*S. brevidens*) were obtained from Potato Introduction Station (Surgeon Bay, WI, USA). The seed tubers were planted in Mix # 5 Plug/LP 5 (Sun Gro Horticulture Canada Ltd., Canada), composed of fine Canadian sphagnum peat moss, fine grade perlite, gypsum, dolomitic lime and wetting agent, in 15 L pots at a depth of 5 cm. Pots were kept in growth chambers at 24 °C with a 16:8 photoperiod under cool white fluorescent light (32 μmol m^{-2} s^{-1}).

Arabidopsis (ecotype Col-0) was used as the wild type as well as the host for ectopic expression of *SbSAMS* gene. Seeds were sown on Murashige and Skoog (MS) agar medium or in Mix # 5 Plug/LP 5 as above for potato. Plants were grown in a growth chamber with 16 h light/8 h dark cycle at 22 °C.

2.2. Vector construction and Arabidopsis transformation

The full length cDNA sequence of *SbSAMS* (GenBank accession number AY635050), was amplified by RT-PCR using cDNA obtained from potato leaf mRNA and primers containing *Bam*HI and *Sac*I sites (F: 5′-CGG GAT CCA TGG AAA CTT TCC TAT TCA C-3′; R: 5′-TCC GAG CTC TTA GTC TTG GGG GTT-3′, Tm 67 °C). After restriction digestion, the PCR fragment was ligated into the pBI121 binary vector (AF485783) (Chen et al., 2003) that contains the CaMV35S promoter, at the sites of *Bam*HI and *Sac*I replacing the GUS gene, resulting in the *SbSAMS* overexpression construct *p35S::SbSAMS* (Supplementary Fig. 1).

Transgenic *Arabidopsis* plants were generated by *Agrobacterium*-mediated transformation using floral dipping method (Zhang et al., 2006). The T_1 seeds were collected and selected for kanamycin (100 mg l^{-1}) resistance on solid MS medium. Kanamycin-resistant lines were analyzed for the presence of transgene by PCR using a 35S promoter-specific forward (5′-GCTCCTACAAATGCCATCA-3′) and a *SAMS*-specific reverse primer (5′-GATAGTGG-GATTGTGCGTCA-3′). The kanamycin-resistant T3 homozygote lines were grown on soil and their phenotypic characteristics were observed throughout the development, and analyzed by semi-quantitative RT-PCR (sqRT-PCR) for the expression of the *SbSAMS* gene using gene-specific primers (Table 1). The samples were amplified using *Ex Taq*™ (TaKaRa, Otsu, Shiga, Japan) under the following conditions: 3 min of initial denaturation at 95 °C and 26 cycles of 30 s at 95 °C and 30 s at 52 °C and 1 min at 72 °C. An internal control was used as the housekeeping gene to normalize the mRNA levels.

Table 1
Primer sequences used in this study.

Gene name	Primer	Primer sequence (5′→3′)
SbSAMS	F	CACCCAGACAAGCTCTGTGA
	R	ACCACTCCTGTCAACCTTGG
SbEF1A	F	CCCCGGACACAGAGACTTTA
	R	GTCAAACCAGTAGGGCCAAA
AtSAM1	F	CCTAGACGAGAAAACCATCTT
	R	CACAATCTT TAG TATCTCCTTG
AtSAM2	F	CCTTGACGACAAAACCATCTT
	R	CAACCCTGTTCCGTAAGTGT
AtSAM3	F	GCCAGTGATCCCAGAGAAATA
	R	CTTTCCTGTTCCATAACTGTC
ATSAM4	F	GCTAAGTACCTTGACGATAAC
	R	GCCTGAAGTCAAATGCTTCC
AtACS6	F	GTTGCGGAAGTAATCGAGGA
	R	ACCGCCTCGTGTCACTAAAG
AtACS10	F	CTTAGCTGATTCGGGAAACG
	R	CCCAACAGTAGAACCCTCCA
AtACS12	F	TATCAGCTCCCCGAGTGTCT
	R	GCCTCCTTCTGTTCCTTTCA
AtSAG12	F	GCTTTAAAACATATGCAAATCTTTCTCTT
	R	CGCAGCTGCCTTGATTCTTGAT
AtRD29A	F	GCGTAACAGGTAAACCTAGAGAA
	R	CTTCTTTGGTGGGATCAGTAACTT
AtABRE	F	CTCAGGCCATGGCCATGAC
	R	GCTGCATTGTTCCACCCATTTT
AtNECD3	F	CTCAGCCCGCCATTATCGTCT
	R	CGGCGTGAACCATACCGTCT
AtCYP707A3	F	CCTAATGTGTTCTTTGCAGCAAAA
	R	GTTCCTGGAAGATTAATCGGC
AtPR-1	F	CAAGATAGCCCACAAGATTATCT
	R	CATAATTCCCACGAGGATCATAG
AtPR-2	F	CAAGTCCATCGGACGTTGTGGCT
	R	CCTGAACCTTCCTTGAGACGGAG
AtPR-5	F	CAGACTTCACTCTAAGGAACAAT
	R	CTGATCCATGACCTTAAGCATGT
AtPDF1.2	F	CATGGCTAAGTTTGCTTCCATCA
	R	GTAAAATACACACGATTTAGCACC
AtActin11	F	GTGTTGGACTCTGGAGATGGTGTG
	R	GCCAAAGCAGTGATCTCTTTGCTC