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Characterization of *S*-adenosylmethionine synthetases in soybean under flooding and drought stresses

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Abstract

Soybean is stress-sensitive crop that exhibits markedly reduced growth under flooding and drought conditions. Three *S*-adenosylmethionine synthetases (SAMs) proteins were identified as flooding and drought responsive proteins in soybean using a proteomic technique. To better understand the role of these SAMs proteins in soybean under flooding and drought stresses, temporal, organ, and stress specificities were examined at mRNA and enzyme activity levels. The activity of SAMs decreased in response to the flooding, however, it was not significantly changed by NaCl, cold, gibberellic acid, and calcium in soybean roots. The activity of SAMs was induced in roots and hypocotyls under drought. The mRNA expression of the *S-adenosylmethionine synthetase* (*SAMs*) family was down-regulated in root tips and roots under the flooding and the drought, and *SAMs 1* and *SAMs 2* were down-regulated in roots under both stresses. A gene *1-aminocyclopropane-1-carboxylate synthase* was up-regulated in root tips, roots, and hypocotyls under drought, however, it was not changed in root tips and roots under the flooding. In addition, *1-aminocyclopropane-1-carboxylate oxidase* was induced in root tips under flooding and drought. These results suggest that SAMs was involved in the response to the flooding and drought and it might affect ethylene biosynthesis in soybean.

Additional key words: 1-aminocyclopropane-1-carboxylate synthase, 1-aminocyclopropane-1-carboxylate oxidase.

Introduction

Soybean is important legume crop, as it is major source of protein and oil for human and animal nutrition (Liu 2008). However, soybean is particularly prone to damage by flooding and drought (Korte *et al.* 1983, Russell *et al.* 1990, Singh *et al.* 2015), the frequency of which continue to increase as consequence of a climate change. Flooding inhibits nutrient uptake (Sallam and Scott 1987) and nitrogen fixation in soybean (Sung 1993) leading to changes in seed composition such as decrease of linoleic/linolenic acids (Van Toai *et al.* 2012), reductions of biomass, taproot length, pod number, and overall plant growth (Githiri *et al.* 2006, Miao *et al.* 2012). In contrast, drought stress decreases photosynthetic rate (Ribas-Carbo *et al.* 2005), water potential of leaves (Liu *et al.* 2004), shoot and root growth (Miao *et al.* 2012), seed yield (Frederick *et al.* 2001) and increases lipid peroxidation and proline content (Alam *et al.* 2010).

Proteomics has been used to investigate organ-specific responses to flooding (Khatoon *et al.* 2012) and drought (Mohammadi *et al.* 2012) in soybean. The findings from these studies suggested that changes in protein abundance are involved in impaired growth of roots, hypocotyls, and leaves under flooding and drought stresses. In addition, Kausar *et al.* (2012) demonstrated that flooding stress induces activity of ascorbate peroxidase which is reactive oxygen species scavenger, whereas exposure to drought induces the opposite effects (Kausar *et al.* 2012). Similarly, it was reported that *S*-adenosylmethionine synthetase (SAMs, EC. 2.5.1.6) activity decreased in soybean under flooding stress,

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Abbreviations: ACC - 1-aminocyclopropane-1-carboxylic acid; GA - gibberellic acid, RT-qPCR - reverse transcription-quantitative polymerase chain reaction; SAM - *S*-adenosylmethionine; SAMs - *S*-adenosylmethionine synthetase.
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but increased in response to drought stress (Oh and Komatsu 2015). These results indicate that SAMs can be potential candidate for studying stress-response mechanisms of soybean.

The SAMs catalyzes biosynthesis of *S*-adenosyl-methionine (SAM) from methionine and ATP (Tabor and Tabor 1984). The SAM serves as methyl donor for pectin methylesterases and *O*-methyltransferase which are involved in pectin and lignin metabolism (Lamblin *et al.* 2001). The SAM is decarboxylated by *S*-adenosyl-methionine decarboxylase to serve as precursor for polyamine biosynthesis (Evans and Malmberg 1989). In plants, ethylene is synthesized from SAM and functions in various physiological processes including stress responses (Yang and Hoffman 1984, Abeles *et al.* 1992). These findings indicate that SAMs is essential for growth and development of plants.

There is evidence that SAMs influences ethylene-mediated inhibition of root growth and alteration of cell wall structures in rice (Fukuda *et al.* 2007). Overexpression of the *SAMs* gene in alfalfa was found to enhance tolerance to cold stress by promoting polyamine oxidation and increasing tolerance to hydrogen peroxide-induced antioxidant protection (Guo *et al.* 2014). An increase in production of SAMs was observed in cold-stressed rice (Cui *et al.* 2005), mechanically wounded *Phaseolus lunatus* (Arimura *et al.* 2002), salt-stressed barley (Witzel *et al.* 2009), and in response to cotton worm feeding in soybean (Fan *et al.* 2012). In contrast, a decreased level of SAMs was observed in soybean exposed to flooding (Hashiguchi *et al.* 2009, Nanjo *et al.* 2010). These results indicate that SAMs are involved in regulation of stress-response mechanisms to flooding and drought in soybean.

In a previous report, three SAMs proteins that are differentially regulated in soybean under flooding and drought stresses were identified using a gel-free proteomic technique (Oh and Komatsu 2015). In the present study, to better understand the role of SAMs in abiotic stress responses, temporal, organ, and stress specificities of these enzymes were analyzed in soybean. The regulation of SAMs in soybean was measured at both mRNA expression and enzyme activity by performing reverse transcription-quantitative polymerase chain reaction (RT-qPCR) and enzyme assays, respectively.

Materials and methods

Plants and treatments: Soybean (*Glycine max* L. cv. Enrei) seeds were sterilized with a 3 % (m/v) sodium hypochlorite solution and rinsed in water. The seeds were sown in silica sand saturated with water in a plastic case (180 × 140 × 45 mm) and grown in a growth chamber at an irradiance of 160 μmol m^{-2} s^{-1} (white fluorescent tubes), a 16-h photoperiod, a temperature of 25 °C, and an air humidity of 70 %. For investigating stress-specific responses, 2-d-old seedlings were exposed to flooding and drought by adding excess water or withholding water, respectively, salt (200 mM NaCl), cold (4 °C), gibberellic acid (10 μM GA_3), or calcium (50 mM $CaCl_2$) for 2 d, and roots were then collected. For examining temporal patterns of stress responses, roots were collected from 2-, 3-, 4-, and 5-d-old soybeans that were treated with the flooding or drought for 0, 1, 2, and 3 d, respectively. For investigating organ specificity of stress responses, root tips, roots, hypocotyls, and cotyledons were collected from 4-d-old seedlings treated with the flooding or drought for 2 d (Fig. 1 Suppl.). All collected samples were either used immediately or stored at -30 °C and then thawed before use. Three independent experiments were performed as biological replicates for each condition.

Phylogenetic analysis: *S-adenosylmethionine synthetase* sequences were retrieved from the soybean genome database *Phytozome v. 9.1* (http://www/phytozome.net/soybean) (Schmutz *et al.* 2010). Phylogenetic analysis of the soybean sequences was conducted using *Clustal W v. 2.1* (http://clustalw.ddbj.nig.ac.jp/top-j.html). Phylogenetic trees were constructed using the *TreeDyn* program (Chevenet *et al.* 2006).

Extraction of RNA and analysis by reverse transcription-quantitative polymerase chain reaction: A portion (100 mg) of frozen samples was ground to powder in liquid nitrogen using a sterilized mortar and pestle, and the total RNA was then extracted using an *RNeasy* plant mini kit (*Qiagen*, Valencia, CA, USA). The extracted RNA was reverse-transcribed to the cDNA using an *iScript* reverse transcription supermix (*Bio-Rad*, Hercules, CA, USA) according to the manufacturer's instructions. RT-qPCR was performed using *SsoAdvanced SYBR Green Supermix* (*Bio-Rad*) on *a MyiQ Single-Color* real-time PCR detection system (*Bio-Rad*). The PCR conditions were as follows: 95 °C for 30 s followed by 45 cycles of 95 °C for 10 s and 60 °C for 30 s. The gene expression was normalized using the *18S rRNA* gene (X02623.1) as internal control. Primers for RT-qPCR were designed using the *Primer3* web interface (http://frodo.wi.mit.edu) (Rozen and Skaletsky 2000, Table 1 Suppl.). Primer specificities were checked by *BLASTN* searches against the *Phytozome* soybean genome database with the designed primers as queries, by melt curve analysis, and by agarose gel electrophoresis of the amplified fragments. The mRNA expression was normalized against the 18S rRNA according to the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen 2001).

Protein extraction and analysis of enzyme activity: A portion (100 mg) of freshly collected samples was homogenized on ice in a buffer consisting of 50 mM Tris-HCl (pH 7.6), 5 mM 2-mercaptoethanol, 10 mM $MgCl_2$, 0.1 mM EDTA, and 2 % (m/v) polyvinylpyrrolidone using a mortar and pestle. The resulting homogenate was centrifuged twice at 10 000 *g* and 4 °C for 15 min and the supernatant was collected and used for enzyme assay. Protein content was determined using the Bradford (1976) method with bovine serum albumin as standard. An enzyme assay reaction mixture consisted of 100 mM Tris-HCl (pH 8.0), 20 mM $MgCl_2$, 150 mM KCl, 2 mM ATP, 5 mM dithiothreitol, and 1 mM methionine. To start the assay, 0.1 cm^3 of the enzyme extract was added to 0.9 cm^3 of the reaction mixture and the activity of SAMs was immediately measured at 340 nm (Kim *et al.* 1992) using a UV spectrophotometer (*DU370*, *Beckman*, Coulter, CA, USA). One unit of SAMs activity was determined as the A_{340} change of 12.44 per min.

Statistical analysis: Statistical significance was evaluated by Student's *t*-test or one-way *ANOVA* followed by Duncan's multiple comparison test (α = 0.05). All calculations were performed using the *SPSS* software (*v. 22.0*, *IBM*, Armonk, NY, USA).

Results

A previous proteomic analysis identified three types of SAMs, SAMs family (Glyma15g21890.1), SAMs 1 (Glyma03g38190.3), and SAMs 2 (Glyma19g40810.1) in soybean exposed to the flooding or drought stress (Oh and Komatsu 2015). The results revealed that these three proteins decrease and increase in response to the flooding and the drought, respectively (Oh and Komatsu 2015). To better characterize SAMs in soybean under the flooding and drought stresses, the stress-, temporal-, and organ-specific changes in SAMs activity were examined (Fig. 1).

To determine stress-specific enzyme activity, the 2-d-old seedlings were exposed to the flooding, drought, 200 mM NaCl (salt), 4 °C (cold), 10 μM GA_3, or 50 mM $CaCl_2$ for 2 d and then roots were collected (Fig. 1*A*). Proteins were extracted and enzyme assays were performed. The activity of SAMs decreased under the flooding stress, but under the other stresses, the enzyme activity was not markedly affected compared to the untreated seedlings (Fig. 1*A*).

To investigate temporal patterns of enzyme activity, the 2-d-old soybean was treated with the flooding or drought for 1, 2, and 3 d, respectively. To perform the drought stress, the mass of the sand in the plastic case was significantly reduced after withholding water for 1 d, and the mass continuously declined during the drought duration. In the untreated soybean, the SAMs activities were not markedly different among the sampling points (Fig. 1*B*). In contrast, the SAMs activity decreased after 1 and 2 d of the flooding, but it increased up to the control level by the third day (Fig. 1*B*). For drought stress, the SAMs activity decreased after 1 d, but increased up to the control level after 2 d. The activity was significantly higher than that detected in the untreated soybean after 3 d (Fig. 1*B*).

To investigate the organ specificity of SAMs activity, the 2-d-old soybean was treated with the flooding or drought for 2 d, and roots, hypocotyls, and cotyledons were collected and then assayed for enzyme activity (Fig. 1*C*). In the roots, the SAMs activity increased during germination, and they had a similar SAMs activity under the drought, but the SAMs activity significantly decreased under the flooding (Fig. 1*C*). In the hypocotyls, the SAMs activity was induced during germination, whereas it declined under the flooding and the drought (Fig. 1*C*). In the cotyledons, there was no significant change in enzyme activity during germination, although the activity decreased under the flooding (Fig. 1*C*).

To determine a relationship among different soybean SAM orthologs and their role in abiotic stress responses, phylogenetic analysis and amino acid sequence alignments were performed. A total of 15 *SAM* sgenes were identified in the *Phytozome* soybean genome database (Fig. 2). The soybean *SAMs* encoded a peptide of 394 - 395 amino acid residues with an estimated molecular mass of 43 053 Da. Phylogenetic analysis using amino acid sequences predicted from the identified gene homologs indicates that *SAMs 1* and *SAMs 2* belonged to the same group, whereas, *SAMs family* belonged to a different group (Fig. 2). The SAMs 1 had 99.75 and 94.18 % amino acid sequence similarities with SAMs 2 and SAMs family, respectively (Fig. 3). The SAMs 2 and SAMs family had a similarity of 94.43 % (Fig. 3). These three SAMs proteins had a hexapeptide motif and a glycine-rich nanopeptide motif (Fig. 3).

SAM is utilized for biosynthesis of ethylene which is converted from a precursor of ethylene, 1-aminocyclo-propane-1-carboxylic acid (ACC) (Fig. 3 Suppl., Wang *et al.* 2002). To further understand whether the induction of SAMs is correlated with ethylene biosynthesis in soybean under the flooding and drought stresses, three *SAMs* genes and two ethylene biosynthetic genes, *ACC synthase* and *ACC oxidase*, were analyzed at the transcriptional level (Fig. 4). The 2-day-old soybean was subjected to the flooding or drought for 2 d, and then root tips, roots, hypocotyls, and cotyledons were collected. These five genes were analyzed by RT-qPCR using the total RNA extracted from the soybean organs. The sizes of the RT-qPCR products were confirmed by agarose gel electrophoresis, and cDNA product sizes were the same as expected (Fig. 4 Suppl.).

The mRNA expression of *SAMs* family was significantly down-regulated in the root tips and the whole roots under the flooding and drought stresses, however, in the hypocotyls it decreased more under the drought compared to the flooding (Fig. 4*A*,*B*,*C*). The expression of *SAMs 1* was down-regulated in the roots, hypocotyls, and cotyledons under the flooding and drought stresses, however, it was up-regulated in the root tips under the drought (Fig. 4*E*,*F*,*G*,*H*). The expression of *SAMs 2* was significantly down-regulated in the roots under both the stresses, whereas, there was no significant changes in the root tips under the drought (Fig. 4*I*,*J*). The expression of *ACC synthase* was significantly up-regulated the in root tips, roots, and hypocotyls under

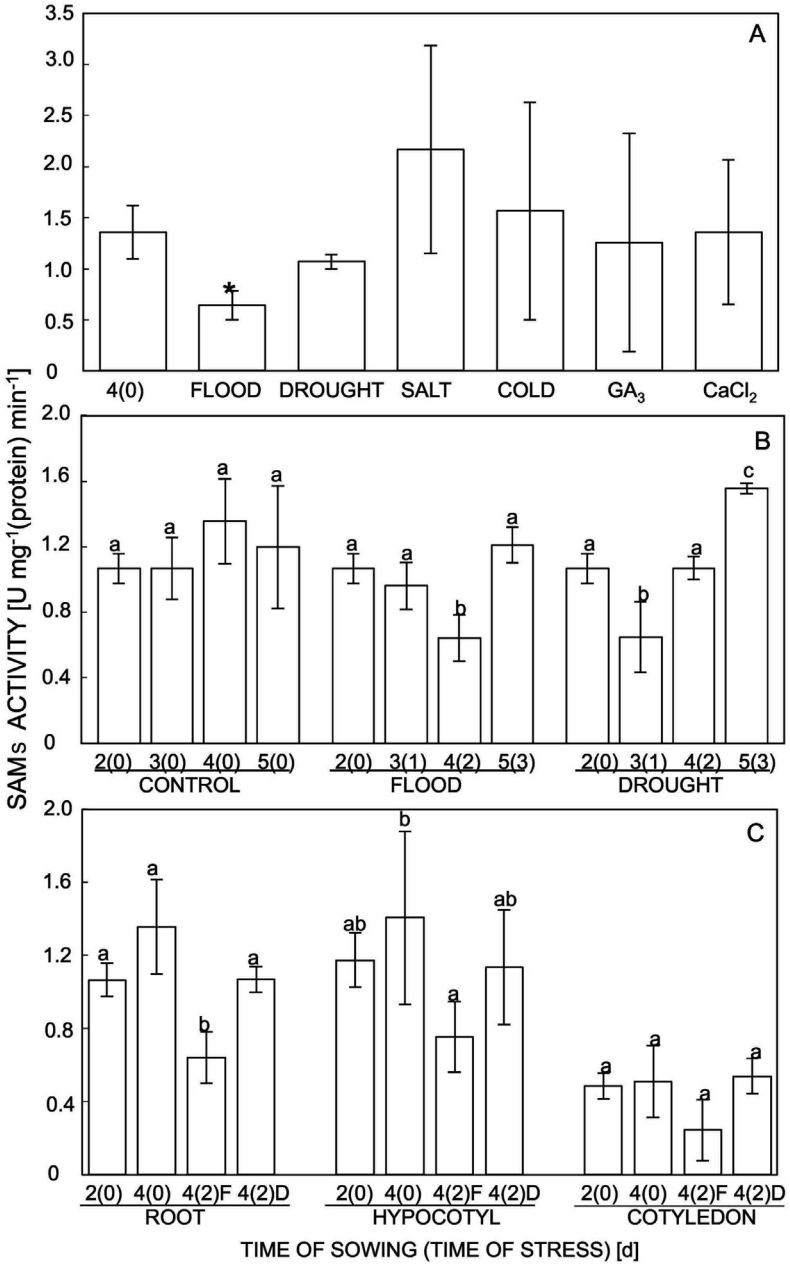

Fig. 1. Stress-, temporal-, and organ-specific activities of *S*-adenosylmethionine synthetases (SAMs) in soybean. To examine stress-specific enzyme activity, 2-d-old soybean was treated with flood, drought, 200 mM NaCl (salt), 4 °C (cold), 10 μM GA_3, or 50 mM $CaCl_2$ (*A*). To investigate temporal changes in enzyme activity, 2-d-old soybean was treated with the flood or drought for 1, 2, and 3 d [3(1), 4(2), and 5(3)], and roots were collected (*B*). To examine organ specificity of enzyme activity, 2-d-old soybean was treated with the flood (F) or drought (D) for 2 d, and roots, hypocotyls, and cotyledons were collected (*C*). Means ± SDs from three independent biological replicates. In *A*, a single *asterisk* indicates significant changes in activity between untreated and treated soybean according to Student's *t*-test ($P < 0.05$). In *B* and *C*, means with different letters indicate significant changes according to Duncan's multiple comparison test ($P < 0.05$). For all experiments, untreated soybean was used as control.

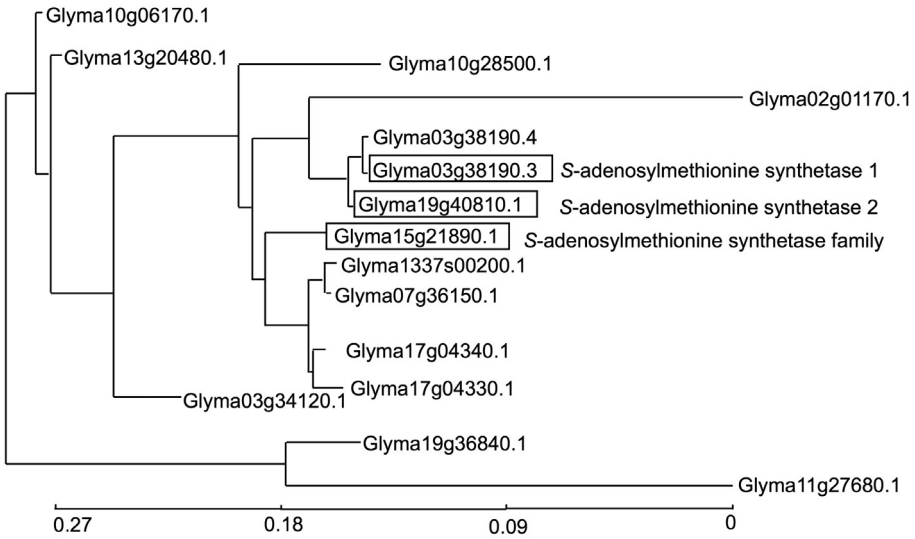

Fig. 2. A phylogenetic tree based on predicted amino acid sequences of *SAMs* in soybean. Phylogenetic analysis was performed using the *Clustal W* software. *Scale bar* corresponds to the number of amino acid substitutions per site. The *outlined* proteins were identified in soybean under flooding and drought stresses (Oh and Komatsu 2015). Protein ID was obtained according to *Phytozome* soybean genome database.

Fig. 3. Amino acid sequence alignments of three types of *SAMs* identified in soybean under flooding and drought stresses. The *outlined* amino acids indicate differences in sequences; the *asterisks* indicate positions which have a single fully conserved residue; the *colons* indicate conservation between groups of strongly similar properties; the *periods* indicate conservation between groups of weakly similar properties, the *boxes* show the sequences of hexapeptide motifs and glycine-rich nanopeptide motifs.

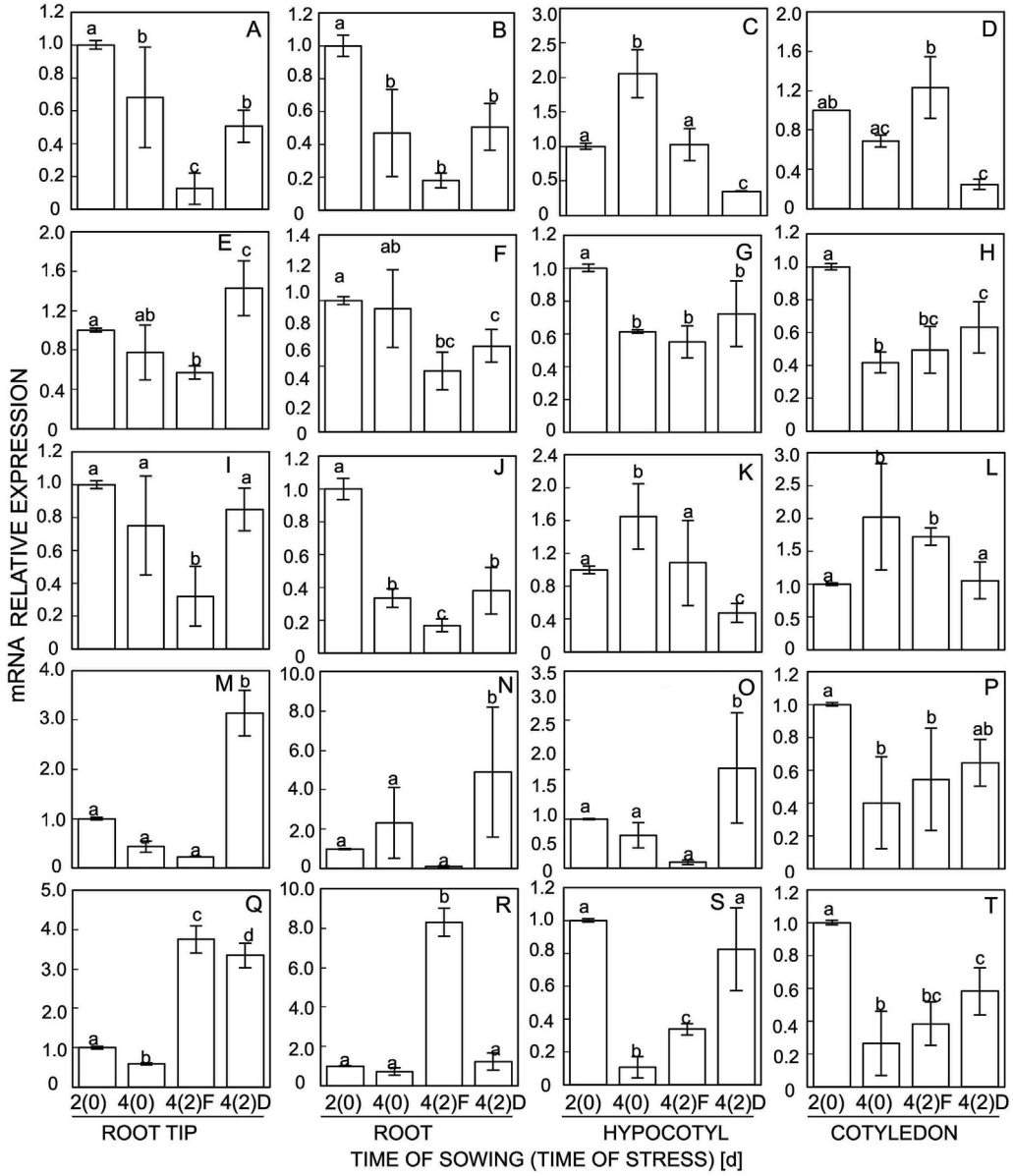

Fig. 4. Effects of flooding and drought stresses on mRNA expression of *SAMs* and *ACC synthase*/*oxidase* genes in soybean root tips, roots, hypocotyls, and cotyledons. 2-d-old soybean was treated with a flooding (F) or drought (D) for 2 d, and total RNA was then extracted from different organs and then analyzed by reverse transcription-quantitative polymerase chain reaction with specific primers. The expressions of *SAMs* family (*A*,*B*,*C*,*D*), *SAM 1* (*E*,*F*,*G*,*H*), *SAM 2* (*I*,*J*,*K*,*L*), *ACC synthase* (*M*,*N*,*O*,*P*), and *ACC oxidase* (*Q*,*R*,*S*,*T*) were analyzed. Relative mRNA expression abundance was normalized against that of the *18S rRNA* gene. Means ± SDs from three independent biological replicates. Means with different letters indicate significant changes according to Duncan's multiple comparison test ($P < 0.05$).

the drought, whereas it was not significantly changed under the flooding (Fig. 4*M*,*N*,*O*). The expression of *ACC oxidase* was significantly up-regulated in the root tips under both the stresses (Fig. 4*Q*).

Discussion

Three SAMs proteins are differentially changed in soybean under the flooding and drought stresses (Oh and Komatsu 2015). In the present study, the activity of SAMs was not significantly changed under the GA_3 treatment (Fig. 1*A*). The amount of *SAMs* transcripts is specifically up-regulated in response to abscisic acid treatments in tomato (Espartero *et al.* 1994). In alfalfa, *SAMs* expression and SAMs protein abundance are

greatly induced in cold-stressed plants after treatment with abscisic acid (Guo *et al.* 2014). Exogenous jasmonic acid strongly induces a *SAMs* gene in *Phaseolus lunatus* leaves (Arimura *et al.* 2002). Kim (2013) also reported that expression of *SAMs* is elevated in GA_3 treatment of barley at an early time point. These results indicate that SAMs activity may be regulated by phytohormones.

All *SAMs* genes are significantly up-regulated in *Catharanthus roseus* under salt stress (Schroder *et al.* 1997). In *Pinus taeda*, *SAMs* gene expression is up-regulated under water deficit (Chang *et al.* 1996). Drought stress leads to accumulation of SAMs in rice (Van Breusegem *et al.* 1994). Expression of *SAMs* and SAMs protein abundance are increased in tomato leaves and roots upon exposure to salinity (Sanchez-Aguayo *et al.* 2004). It was reported that the amount of *SAMs* trancripts is specifically up-regulated in response to NaCl and mannitol in tomato (Espartero *et al.* 1994). However, expression of *SAM* in *Pinus banksiana* is not affected by heat, cold, or anoxia (Mayne *et al.* 1996). In the present study, the SAMs activity was significantly decreased under flooding but not changed under drought, suggesting that flooding tolerance of soybean might be reduced through the dampen activity of SAMs.

In the soybean exposed to the flooding, the activity of SAMs was gradually decreased after being exposed to flooding for 2 d, but it was increased by flooding for 3 d compared to the untreated soybean. The activity of SAMs was significantly decreased and increased after being exposed to drought for 1 and 3 d, respectively (Fig. 1*B*). In barley, the transcripts of *SAMs* genes in developing kernels predominantly accumulate during the first 10 d after fertilization (Kim 2013). Kim (2013) also found that barley *SAMs* genes are induced within the first 12 h after wounding, NaCl, abscisic acid, and spermidine treatments. Expression of a ginseng *SAMs* gene is also up-regulated during the first 12 h of salt stress, but it is then down-regulated between 24 and 72 h (Pulla *et al.* 2009). Activity of SAMs and *SAMs* transcription in mature leaves of alfalfa are highly induced between 8 - 48 h and 8 - 96 h, respectively, after cold treatment (Guo *et al.* 2014). Taken together, these results indicate that *SAMs* expression or SAMs activity are related to a variety of stresses and SAMs is involved in the early stage of soybean response to flooding and drought.

The SAMs activity increased in roots, hypocotyls, and cotyledons during germination without the stresses, and it markedly decreased in roots under the flooding (Fig. 1*C*). In *Arabidopsis* stems, roots, and calli, expression of the *SAMs* gene is approximately 20-fold higher than in leaves, seeds, pods, and inflorescences (Peleman *et al.* 1989a). An increased *SAMs* gene transcription corresponds to a 10- to 20-fold higher SAMs activity in stems than in leaves (Peleman *et al.* 1989b). Similarly, it was reported that a *SAMs 1* gene is more expressed in roots as compared to shoots of lodgepole pine (Lindroth *et al.* 2001) and ginseng (Pulla *et al.* 2009). The transcripts of barley *SAMs* are more accumulated in grains, stems, and leaves as compared to roots (Kim 2013). In contrast, the results from the present study demonstraste that SAMs had higher activities in roots and hypocotyls compared to cotyledons. These results suggest that SAMs might play specific roles in soybean roots.

Above mentioned studies indicate that *SAMs* has its homologous genes which have different or similar expression trends in temporal, organ, and stress responses. The *SAMs* genes were isolated from *Arabidopsis* (Peleman *et al.* 1989a), poplar (Van Doorsselaere *et al.* 1993), and *Lycoris radiata* (Li *et al.* 2013). The amino acid sequences of SAMs display a high similarity among these plants indicating that this enzyme is highly conserved among plants. To further understand the similarities among three SAMs proteins identified in soybean (Oh and Komatsu 2015), a phylogenetic analysis was performed (Fig. 2). As confirmed by the high similarity in amino acid sequences (Fig. 3), the three SAMs proteins were closely related isoforms. In plants, the amino acid sequence of SAMs contains two conserved motifs (Bairoch 1993): a hexapeptide motif that is thought to bind to the adenine moiety of ATP, and a glycine-rich nanopeptide motif consisting of a P-loop-like sequence, that is involved in binding the triphosphate of ATP. These two motifs were found in the amino acid sequences of the three SAMs in soybean demonstrating that soybean SAMs is a highly conserved enzyme.

S-adenosylmethionine is intermediate in the ethylene biosynthesis (Roeder *et al.* 2009). The ethylene biosynthesis consists of two enzymatic steps, the first step is synthesis of ACC from *S*-adenosylmethionine by ACC synthase, and the second step is conversion of ACC to ethylene by ACC oxidase (Wang *et al.* 2002) (Fig. 3 Suppl.). In addition to ACC, ACC synthase produces 5'-methyl-thioadenosine which must be recycled back into the methionine cycle to provide an adequate supply of methionine as substrate for continuous production of ethylene (Bleecker and Kende 2000). An accelerated synthesis of ethylene demands a greater supply of *S*-adenosylmethionine because the main control step in ethylene production is conversion of *S*-adenosyl-methionine to ACC. Induction of SAMs during stress increases the amount of *S*-adenosylmethionine, which positively affects ethylene biosynthesis (Roeder *et al.* 2009). In soybean, it has been reported that ACC synthase (Khan *et al.* 2014) and ACC oxidase (Komatsu *et al.* 2009, Nanjo *et al.* 2010) respond to flooding. It is necessary to confirm a relationship between soybean SAMs activity and ethylene biosynthesis under flooding and drought stresses.

It was reported that ethylene biosynthesis is regulated by *SAMs* expression in mustard (Lim *et al.* 2002). In *P. lunatus* leaves, expression of *SAMs* and *ACC oxidase* was significantly induced by pest infestation and mechanical wounding (Arimura *et al.* 2002). Expression of *ACC synthase* is up-regulated in transgenic

Arabidopsis overexpressing a *SAMs* gene indicating that SAM produced in the transgenic plants is dominantly converted to ethylene rather than to polyamines (Kim *et al.* 2015). Conversion of ACC to ethylene by ACC oxidase is oxygen dependent process (Kende 1993). Ethylene biosynthesis is considered to be limited at the activity of ACC oxidase rather than of ACC synthase in *Rumex palustris* during submergence (Vriezen *et al.* 1999). The ACC oxidase accumulates under flooding stress because of flooding-induced oxygen deprivation, thereby resulting in inhibition of ethylene production (Vriezen *et al.* 1999). In the present study, the expression of *ACC oxidase* was significantly induced in root tips under the flooding and the drought and was up-regulated in roots under the flooding (Fig. 4). These results suggest that SAMs might be involved in ethylene production in soybean root tips under the flooding and the drought.

Oh and Komatsu (2015) identified that three SAMs proteins are differentially expressed in soybean under flooding and drought stresses. In the current study, the activity of SAMs decreased during the early stage of the flooding stress, but increased in the later stage of the drought stress indicating that SAMs may be involved in the response to these stresses in soybean. The SAMs activity was not the same in the different organs as well as in response to the stresses. The expressions of *SAMs family*, *SAMs 1*, and *SAMs 2* were down-regulated in roots under the flooding and the drought. In addition, in root tips, *ACC synthase* was down-regulated under the flooding, but it was up-regulated under the drought, whereas *ACC oxidase* was induced under both the stresses. Taken together, these results suggest that SAMs might be an important enzyme regulating biosynthesis in soybean.

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