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# Activation of Barley S-Adenosylmethionine Synthetase1 Gene Promoter in Response to Phytohormones and Abiotic Stresses

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## Abstract

Barley S-adenosylmethionine synthetase1 gene, which was differentially expressed in seed development of extra early barley, was regulated by the phytohormones and abiotic stresses. In order to identify the regulation regions which were involved in transcriptional control of the phytohormones and abiotic stresses, we isolated 1459 bp fragment of *HvSAMS1* gene promoter using genome walking strategy and deletion series were constructed. Deleted upstream fragments (-1459, -1223, -999, -766, -545, -301 bp) were fused to the GUS reporter gene and evaluated via *Agrobacterium*-mediated transient expression assay. Increased *GUS* activity of *HvSMAS1* promoter -301/GUS construct under each of NaCl, $GA_3$, ABA and ethylene application was found. However, GUS activity was negligible in the leaves transformed with the *HvSMAS1* promoter (-1459, -1223, -999, -766 and -545)/GUS constructs. No significant induction of GUS activity was observed for the ethionine and spermidine treatments.

In order to locate promoter sequence of the *HvSAMS1* gene that was critical for the activation of gene expression, deletion and addition promoter derivatives (+, includes 43 bp of 5' ORF) of the *HvSAMS1* gene fused to the GUS reporter gene were applied. The tobacco leaves which harbored the additional *HvSAMS1* promoter (-1459+, -1459 to -546, -545+ and -301+)/GUS construct did not significantly induce GUS activity as compared to the *HvSAMS1* promoter (-1459, -545 and -301)/GUS constructs under each of NaCl, ABA and $GA_3$ treatment. However, the GUS activity was high in the tobacco leaves which harboring the -211 to -141 regions of the *HvSAMS1* promoter. This result suggested that *HvSAMS1* gene expression might be regulated by this region (from -211 to -141)

Key words: Barley, *eam10, HvSAMS1,* Promoter

## Introduction

Early stage of cereal grain development is considered as a crucial stage for further progress of storage material transfer and accumulation. Plant S-adenosylmethionine (AdoMet) is known as the methyl group donor, involved in transmethylation of proteins, nucleic acids, polysaccharides and fatty acids (Schlenk and DePalma, 1957). AdoMet is produced by S-adenosylmethionine synthetase (SAMS) release of ATP and L-methionine (Cantoni, 1953). Furthermore, decarboxylated SAM serves as an amine propyl donor in the synthesis of polyamine (Kumar et al., 1997), and SAM is a precursor in the synthesis of ethylene (Yang and Hoffman, 1984).

SAMS is a well conserved enzyme in plant, animal and bacteria. *SAMS* has been isolated from a variety of organisms (Elleuch et al., 2001). It suggested that some of the *SAMS* might be specifically regulated by hormonal treatments (Mathur et al., 1991) and environmental factors (Espartero et al., 1994). Differential regulation of expression of the *SAMS* genes was reported in tomato (Schröder et al., 1997) and periwinkle (Eapartero et al., 1994).

*SAMS* genes under the control of the CaMV 35S constitutive promoter were introduced into *Arabidopsis thaliana* (Peleman et al., 1989a), *Papaver somniferum* (Belny et al., 1997) and *Nicotiana tabacum* (Belbahri et al., 2000). The promoter *of Arabidopsis thaliana* SAMS genes was introduced into tobacco and poppy *via Agrobacterium*-mediated (Peleman et al., 1989b).

In order to gain an insight into the functional regulatory mechanisms of interesting gene expression, the gene promoters were isolated and characterized from several plant species (Hong et al., 2005). In general, *in vivo* characterization of plant promoter was monitored by measuring activities of reporter gene that was fused with promoters in transgenic plants. This process is labor-intensive, time-consuming and

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may generate significant diversity due to insertional positions. To overcome some of these disadvantages, transient expression assay has been developed based on *Agrobacterium*-mediated transformation of tobacco leaves (Yang et al., 2000).

Recently the relationship between gene expression and *cis*-elements draws growing attention. GCC box (AGCCGCC) was found in the promoters of many pathogen-responsive genes (Hong et al., 2005) and has been known to be an ethylene-responsive element. GCC box played an important role in regulating jasmonate-responsive gene expression (Brown et al., 2003).

RAV1 and RAV2 were transcription factors, which possessed two distinct DNA-binding domains, AP2 and B3, and were found only in higher plant species (Kagaya et al., 1999). RAV1 bound to the CAACA and CACCTG motifs (Kagaya et al., 1999) which were essential for the RAV1 protein to bind to a specific target gene in pepper (Sohn et al., 2006).

In addition, *cis*-acting elements, normally referred as GA responsive elements (GAREs), have been identified in analysis of genes such as $\alpha$-amylase (Skriver et al., 1991) and *SPY* (Robertson et al., 1998) in barley.

Recently, a gibberellin induced *EPB1* gene, a cysteine proteinase responsible for the degradation of seed endosperm storage proteins in barley, was found that it had pyrimidine box that was a factor required for GA induction (Cercós et al., 1999).

The significant roll of *HvSAMS1* promoter was investigated by the differential regulation of the expression in response to phytohormones and abiotic stresses using *Agrobacterium*-mediated transient assay.

## Materials and Methods

### Plant materials and growth condition

Seeds of barley germplasm 'GSHO 2504' were germinated in a petri-dish and was transplanted to the soil and allowed to grow at 25℃ /18℃(day/night) for 4 weeks. Tobacco (*Nicotiana tabacum*, cv. *Xanthi-nc*) seeds were sown in a soil mix (loam soil/perlite/vermiculite, v/v/v, 3/1/1) and allowed to grow in a growth chamber under a controlled environment at 24+ 2℃ and illuminated with 16 h photoperiod. *Agrobacterium*-mediated transient assay was conducted at 6-leaf stage of tobacco plants.

### Treatment with phytohormones and abiotic stresses

In order to characterize the induction of promoter activity, tobacco plants were treated with ethylene, abscisic acid (ABA), ethionine, NaCl, gibberellic acid ($GA_3$) and spermidine. For ethylene treatment, the tobacco plants were covered with 5 liter glass beaker. After the beaker was tightly sealed, the ethylene gas was injected inside to make a concentration of 5$\mu\ell/\ell$ . For each ABA, ethionine, NaCl, $GA_3$ and spermidine application, the tobacco plants were sprayed with 100 $\mu$M NaCl and 100$\mu$M each ABA, ethionine, $GA_3$ and spermidine, respectively.

For the induction of *HvSAMS1*, barley seedlings were treated as described above except ethylene concentration (20$\mu\ell/\ell$).

### RNA isolation and Northern blot analysis

Total RNA was extracted using Trizol reagent (Invitrogen). Total RNA (10$\mu$g) isolated from each treated barley was separated on 1% formaldehyde agarose gel, and then transferred onto Megna charge nylon membrane (Osmotics). The gene specific probes were labeled with full length cDNA as a template containing 5′ and 3′ untranslation regions. The methods of hybridization and detection were followed as described by Jang et al. (2003).

### Isolation of the *HvSAMS1* gene promoter

Genomic DNA was isolated from the fresh leaf tissues (1g) of barley germplasm "GSHO 2504" as described by Seo et al. (1997). The differential hybridization (DH) method was described by Lee et al. (2006) with Digoxygenin (DIG)-labeled probe. The universal Genome walker™ Kit (Clonetech) was used to isolate the *HvSAMS1* gene promoter region. The isolated genomic DNA was digested with seven blunt end-forming restriction enzymes (*Dra*I, *Eco*RI, *Pvu*II, *Stu*I, *Bbr*PI, *Sma*I and *Sna*BI). The adaptor DNA containing AP1 and AP2 primer binding sites was linked to both ends of the restricted DNA fragments at 16℃ overnight. AP1 (5′-GTAATACGACTCACTATAGGGC-3′) and AP2 (5′-ACTATAGGGCACGCGTGGT-3′) primers were used for PCR amplification by pairing them with the two *HvSAMS1* gene specific primers. The gene specific primers, HvSAMS 3′-1 (5′-CCGAAGACCATGACCATGTTGGTCTTGGTG-3′) and HvSAMS 3′-2 (5′-GTTCACGGACTCGGAAGTGAAGAGGAAC-3′), were designed based on the *HvSAMS1* cDNA sequence. The nested PCR was performed with AP1 and HvSAMS 3′-1, AP2 and HvSAMS 3′-2. The PCR products were cloned into the pGEM easy vector (Promega) and sequenced. DNA sequencing was performed on an ABI PRISM 310 Genetic analyzer (Perkin Elmer) and analyzed with the BLAST alignment (Altschul et al., 1997), PLACE Web Signal Scan (Higo et al., 1999) and PlantCARE (Lescot et al., 2002).

### Construction of deletion series fused with GUS

The construction of 5′ deletion series was performed with promoter region specific primers by PCR amplification. Deletion fragment of -1,459 bp, -1,223 bp, -999 bp, -766 bp, -545 bp, -301 bp, -211 bp, -141 bp to -1 were prepared using forward and reverse primers.

Forward primers are

- 1,459 (K800Promo I F); 5′-TACAAGCTTGGTGATAGCAAAATGTGATG-3′,
- 1,223 (K800Promo II F); 5′-TACAAGCTTGGAGACGAGGAAAATGCGAATC-3′,
- 99 (K800Promo III F); 5′-TACAAGCTTCCAGCAACACTCACGCACGAC-3′,
- 766 (K800Promo IV F); 5′-TACAAGCTTCTACTCCCTCTGTAAAGAACG-3′,
- 545 (K800Promo V F); 5′-TACAAGCTTCTTTCTGCGTCGTCGTCACGGCA-3′,
- 301 (K800Promo VI F); 5′-TACAAGCTTGACTCTCGTCGCTCCTTCC-3′,
- 211 (K800Promo VI-2 F); 5′-TACAAGCTTGGGACCTGTTTGTTATTTCTCTTG-3′,
- 141(K800Promo VI-3 F); 5′-TACAAGCTTGCTTATTATTCCAGGCTGTGCGGTA-3′

and the reverse primer is

-1 ( K800Promo R); 5′-TTAGGATCCTTCTTTGGTGGTAGAGATGC-3′.

Other reverse primers that contain N-terminal cDNA sequence (K800Promo R1: 5′-TAGGATCCGTTCACGGTACTCGGAAGT-3′) and -569 to -546 (K800Promo R2); 5′-TTAGGATCCCGGAGGAACAAAAGATCCATAGAA-3′ were used. The forward primers contained a *Hin*dIII site and the reverse primers contained *Bam*HI site (restriction sites were underlined).

The resultant constructs were digested with *Hin*dIII and *Bam*HI, and was then replaced with the CaMV 35S promoter in pBI121 vector (Clontech). The PCR products were cloned into the pGEM easy vector (Promega) and were confirmed by DNA sequencing. The con-

structs were introduced into the *Agrobacterium tumefaciens* strain, GV3101, *via* freeze-thaw method as described by Chen et al. (1994).

### Transient expression assay of plants mediated by *Agrobacterium*

An *Agrobacterium*-mediated transient expression assay was followed as described by Yang et al. (2000). *Agrobacterium tumefaciens* strain GV3101 containing serially deleted promoter/*GUS* chimeric constructs was grown on a YEP medium supplemented with the antibiotics kanamycin (50μg/ml), refampicin (50μg/ml), gentamycine (25μg/ml). *Agrobacterial* cells were cultured in 50 ml of YEP medium overnight at 28℃. The *Agrobacteral* cells were collected by centrifuge at 12,000g for 10min. After removal of supernatant, cell pellet was suspended in infiltration medium (10mM MES, pH 5.7, 10mM $MgCl_2$, 200μM acetosyringone) and adjusted to an $OD_{600}$ of 0.8 to infiltrate into the tobacco leaves. After infiltration of the *Agrobacterium* using a needleless syringe, the tobacco plants were placed in a moist chamber at 28℃ for 48h followed by application of phytohormone and abiotic stress treatments.

### GUS activity measurement

The GUS activity in the *Agrobacterium*-mediated transiently expressed tobacco leaves was measured as described by Jefferson et al. (1987). The tobacco leaf tissues were ground in a mortar using liquid nitrogen, and the tissue powder was transferred to a 1.5ml microfuge tube. After addition of 1 ml of extraction buffer (50 mM $NaH_2PO_4$, pH 7.0, 10mM EDTA, 0.1% Triton X-100, 0.1% sodium laurylsarcosine, 10mM β-Mercaptoethanol) was added and mixed. After centrifugation at 12,000g for 10 min at 4℃, the supernatant was transferred to a new 1.5 ml tube. Total 1 ml volume containing 1 mM 4-methylumbelliferyl-β-D-glucuronide (MUG) (Sigma) in an extraction buffer supplemented with 0.1 ml aliquot of the protein extract supernatants was prepared for fluorogenic reaction. GUS activity was normalized to protein concentration in each of the crude extracts, and was calculated as the pmol of 4-methylumbelliferone (4-MU) produced per minute, per milligram of soluble protein. The protein contents of the sample extracts was determined using bovine serum albumin (BSA) as a standard (Bradford, 1976).

## Results

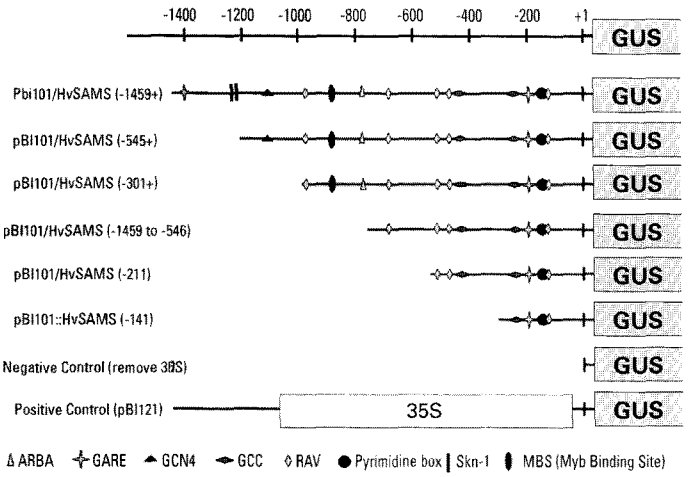
### Isolation of the *HvSAMS1* gene promoter

In order to isolate genes expressed during early stage of grain development, differential hybridization (DH) method using different kernel developmental stages (-3 DAF, 3 DAF and 13 DAF) was conducted. Randomly selected 800 barley cDNA clones were employed in the differential hybridization. Individual 65 cDNA clones showed a significant degree of homologies to the sequences registered in NCBI. One clone whose transcript was high at 3 DAF and showed highly homology to S-adenosylmethionine synthetase was identified and designed as *HvSAMS1* (*Hordeum vulgare S-AdenosylMethionine Synthetase 1*).

In order to understand mechanisms responsible for the regulation of *HvSAMS1* gene expression in response to phytohormones and abiotic stress applications, the genome walker strategy was used to isolate the *HvSAMS1* gene promoter region from the genomic DNA of barley. Since *HvSAMS1* contained no intron region in its ORF, a pair of nested gene specific primers was designed using the *HvSAMS1* cDNA sequence. The amplified *HvSAMS1* gene promoter region was 1459 bp in length (Fig. 1). *Cis*-acting elements that were putatively involved in the stress-response and seed specific expression were identified using PLACE (http://www.dna.affrc.go.jp/PLACE/) and PlantCARE (http://bioinformatics.psb.ugent.be/webtools/plantcare/html/) (Fig. 1).


**Fig. 1.** Nucleotide and deduced amino acid sequences of the *HvSAMS1* gene, including 1459 bp upstream of the ATG. The nucleotide of the ATG translation initiation codon was assigned as position 1 in the nucleotide sequence, and the nucleotide positions upstream of position 1 were presented as negative numbers.

A TATA box (TTATTT) was recognized at -30. There were one ABRE, two GAREs, two GCC boxes, one GCN4 motif, one MYB binding site, two Skn-1 motifs, five RAVs, one pyrimidine box and two CGTCA motifs within 1459 bp upstream of *HvSAMS1* (Fig. 1). In order to analyze the molecular mechanisms in response to phytohormones and abiotic stresses regulation, a series of 5′ deletions from the *HvSAMS1* promoter region were prepared. Six promoter deletion derivatives (-1459, -1223, -999, -766, -545 and -301 bp from the translational initiation site of the *HvSAMS1* gene) fused to the *GUS* gene were constructed (Fig. 2). The induction of GUS activity of each construct by the exogenous application of the growth phytohormones and abiotic stresses were analyzed using a quantitative fluorometric assay.




**Figs. 2.** Schematic diagram of different *HvSAMS1* promoter/GUS fusions. The different length promoter fragments were inserted into pBI 121, which was removed CaMV 35S. The DNA construct sequences were shown to describe the fusion of the GUS gene to the *HvSAMS1* promoter. The putative cis-acting elements were represented by symbols.

## Activation of the *HvSAMS1* gene by phytohormones and abiotic stresses

In order to understand transcript profiling of the *HvSAMS1* gene in response to phytohormones and abiotic stresses Northern blot analysis of treated barley leaves was conducted. *HvSAMS1* gene expression was not detected in plants treated with ethionine, a methionine analog as well as in the mock treated plants (Fig. 3). The transcripts of *HvSAMS1* were detected in response to each NaCl, ethylene, ABA, $GA_3$ and spermidine treatment.

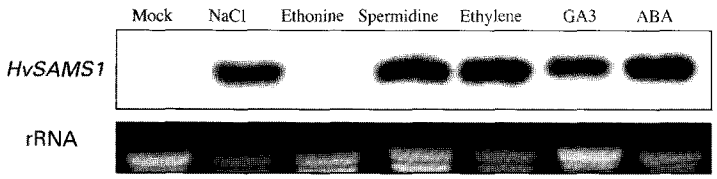



**Fig. 3.** Northern blot analysis of the *HvSAMS1* gene with elicitor treatment. Total RNA (10μg per sample) of leaf was fractionated on a 1% denatured agarose gel.

## Activation of the *HvSAMS1* promoter by phytohormones and abiotic stresses

In order to identify the promoter sequence of *HvSAMS1* gene, expression analysis of six chimeric *HvSAMS1* promoter/GUS constructs in tobacco leaves treated with NaCl, ethylene, ABA, $GA_3$, ethionine and spermidine was conducted. GUS activity driven by the *HvSAMS1* promoter (-301)/GUS construct was high as compared to the negative control, but wasnot found in the leaves harboring the *HvSAMS*1 promoter (-1459, -1223, - 999, -766 and -545)/GUS constructs (Fig. 4). GUS induction patterns as the results of ethylene, ABA and $GA_3$ treatment were similar to those observed in the tobacco leaves treated with NaCl. No significant induction of GUS activity was observed in the tobacco leaves treated with each of ethionine and spermidine. In each treatment of NaCl, spermidine, $GA_3$ and ethionine, GUS activity was lower in *HvSAMS1* promoter (-545)/GUS construct than *HvSAMS1* promoter (-766 and -301)/GUS constructs.

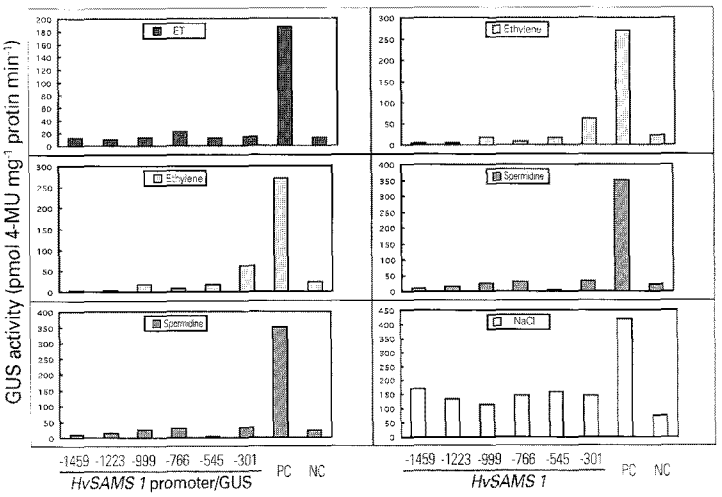



**Fig. 4.** Effects of several abiotic elicitors on the transient expression of the *HvSAMS1* promoter/GUS gene in tobacco leaves. The activity of 5′ deletions of the *HvSAMS1* promoter was determined in tobacco plants which had been treated with 100 mM NaCl, 100 μM $GA_3$, 100 μM ABA, 100 μM spermidine, 100 μM ethionine, 20 μl/l ethylene. The GUS activity, analyzed fluorometrically, was quantitatively displayed in pmol 4-MU $mg^{-1}$ protein $min^{-1}$.

## Deletion analysis of *HvSAMS1* promoter

Promoters of further deletions or additional 5′ ORFs were designed to determine which of these regions have an essential role in the gene expression in response to growth hormones and abiotic stress treatments (Fig. 5).

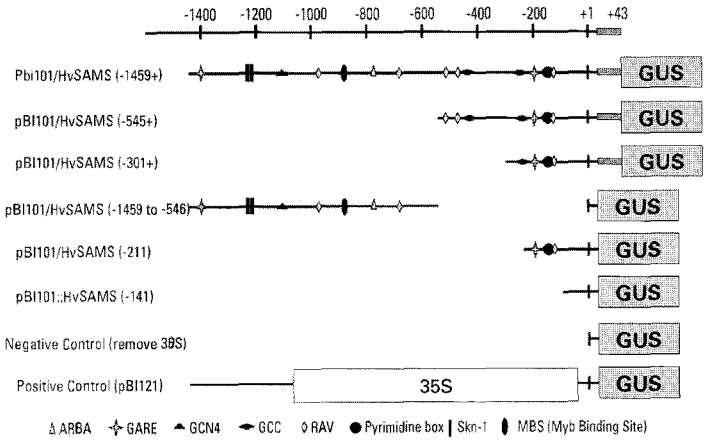
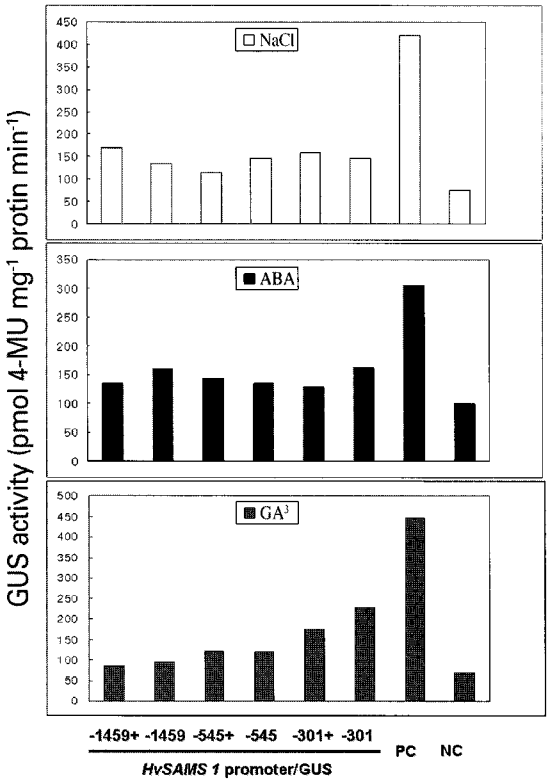



**Fig. 5.** Schematic diagram of additional and deletional *HvSAMS1* promoter/GUS fusions. The different length promoter fragments were inserted into pBI 121, which was removed CaMV 35S. The DNA construct sequences were shown to describe the fusion of the *GUS* gene to the *HvSAMS1* promoter. The putative *cis*-acting elements were represented by symbols.

The first type of constructs, *HvSAMS1* promoter and N-terminal region contained 43 bp downstream regions from translation initiation site [-1459+ (-1459 to +43), -545+ (-545 to +43), -301+ (-301 to +43)] were fused to GUS reporter gene. The second type, -1459 to -546, was constructed with fusion to GUS reporter gene. The tobacco leaves which harbored the additional *HvSAMS1* promoter (-1459+, -1459 to -546, -545+ and -301+)/GUS construct did not significantly induce GUS activity as compared to the *HvSAMS1* promoter (-1459, -545 and -301)/GUS constructs in the leaves treated with each of NaCl, ABA and $GA_3$ (Fig. 6).




**Figs. 6.** Effects of several abiotic elicitors on the transient expression of the *HvSAMS1* promoter/GUS gene in tobacco leaves. The activity of 5′ deletions of the *HvSAMS1* promoter was determined in tobacco plants which had been treated with 100 mM NaCl, 100 μM $GA_3$, 100 μM ABA, 100 μM spermidine, 100 μM ethionine, 20 μl/l ethylene. The GUS activity, analyzed fluorometrically, was quantitatively displayed in pmol 4-MU $mg^{-1}$ protein $min^{-1}$.

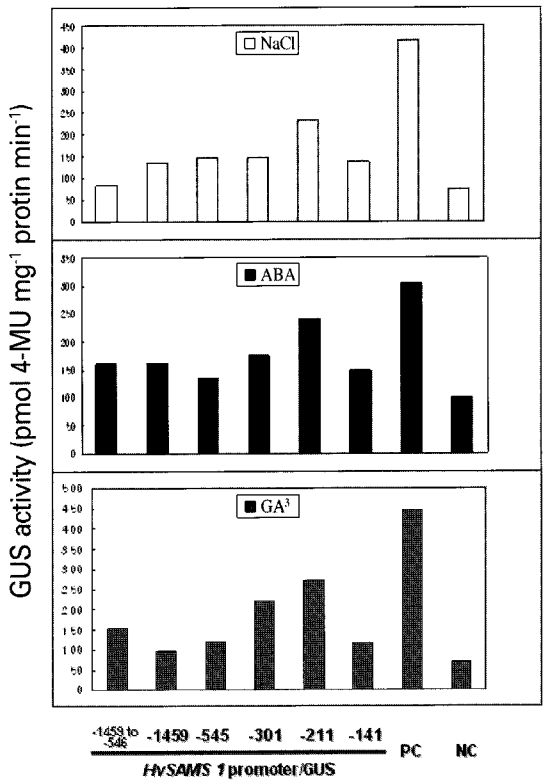



**Fig. 7.** Induction of the GUS activity driven by the *HvSAMS1* deletional promoters in the tobacco leaf tissues. The activity of 5′ deletions of the *HvSAMS1* promoter was determined in tobacco plants which had been treated with 100 mM NaCl, 100 μM $GA_3$, 100 μM ABA, 100 μM spermidine, 100 μM ethionine, 20 μl/l ethylene. The GUS activity, analyzed fluorometrically, was quantitatively displayed in pmol 4-MU $mg^{-1}$ protein $min^{-1}$.

Based on our promoter analysis, we confirmed that the *HvSAMS1* specific promoter region, extending from -301 to -1 bp, was a critical region for the induction of the *HvSAMS1* gene as the result of exposure to exogenous growth hormones and abiotic stresses (Fig. 4). To determine which specific promoter sequence of the *HvSAMS1* gene is truly critical for the activation of gene expression, we constructed the third type promoter derivatives (-301, -211 and -141) of the *HvSAMS1* gene fused to the GUS reporter gene (Fig. 5). These three different types of chimeric constructs were tested for the induction of GUS expression in the tobacco leaves with NaCl, ABA and $GA_3$ treatment. In the leaves treated with NaCl, ABA and $GA_3$, higher GUS activity was detected in leaves transformed with the *HvSMAS1* promoter (-211)/GUS construct than in the leaves harboring the *HvSMAS1* promoter (-301 and -141)/GUS constructs (Fig. 7).

## Discussion

### Spectral analysis

The barley germplasm 'GSHO 2504', known as an early maturity 10 (possessing *eam10* gene), has been incorporated in barley breeding programs for early maturity (Börner et al., 2002). In this study, we isolated the *HvSAMS1* gene at 3 DAF barley grain from the 'GSHO 2504' using DH methods. We also isolated the 1459 fragment of *HvSAMS1* promoter region and analyzed the promoter region *via* transient expression assay.

In the deletion analysis of the *HvSAMS1* gene promoter, the -301/*GUS* construct up-regulated GUS expression in response to each of NaCl, ethylene, ABA and $GA_3$ treatment (Fig. 4). However, GUS activity was increased with the deletion of -211 region of the *HvSAMS1* compared to that of -301 region (Fig. 5) This result indicated that the *cis*-acting elements affected the *HvSAMS1* gene expression under NaCl, ABA and $GA_3$ treatment. Negligible GUS expression in response to NaCl, ABA and $GA_3$ treatment was found in -141/*GUS* construct. This suggested that the minimal *cis*-element sequence was necessary for the expression in response to NaCl, ABA and $GA_3$ treatment and this sequence might be located between -211 and -141 of the *HvSAMS1* promoter. One GARE, one pyrimidine box, and one RAV site were present within the -211 region of the *HvSAMS1* promoter.

The *cis*- acting elements involved in GA induced gene expression have been previously studied. GARE, Box I, and pyrimidine box were reported to be the regulatory elements on the $GA_3$ induction of *EPB-1* expression (Cercós et al., 1999). They also showed that GARE was coupled with the pyrimidine box to form a GA response complex. As the -211 region of the *HvSAMS1* promoter has GARE and pyrimidine box, the -211 region might be significantly response to the $GA_3$ treatment.

ABA was a known antagonistic regulator of GA, but GUS activity of -211/*GUS* construct was increased in the ABA treatment. This result suggested presence of other element(s) that resided -211 region and this element(s) might be required for expression under exogenous ABA. *HvSPY* gene modulated the transcriptional activities of two hormonally regulated promoters in GA and ABA (Robertson et al., 1998). Cercós et al. (1999) showed that a constitutive expression of a

transcription factor, GAMyb, leaded to the increase of the *EPB-1* promoter/*GUS* activity in GA and ABA treatment. The *EPB-1* promoter could be co-ordinately regulated by GA and ABA through GAMyb. These provided a possibility for the presence of unknown ABA response *cis*-acting elements and/or *trans*-acting elements between -211 and -141.

The transcription factor, RAV1 possesses AP2 and B3 domains that are involved in different biological effects (Sohn et al., 2006). No other plant transcription factors, except for RAV, harbor two or more distinct types of DNA-binding domains (Kagaya et al., 1999). Using binding site selection analysis, the AP2 and B3 domains of RAV1 were bound to RAV1 (CAACA) and RAV2 (CACCTG) motif, respectively (Kagaya et al., 1999). Overexpression of pepper RAV1 gene in transgenic *Arabidopsis* induced the constitutive expression of the *Arabidopsis* PR gene. These transgenic plants also exhibited the enhanced resistance to osmotic stress (NaCl) (Sohn et al., 2006). GUS activity was high in the tobacco leaves which harboring the -211 to -141 regions of the *HvSAMS1* promoter. This result suggested that *HvSAMS1* gene expression might be regulated by the cis-acting element, RAV, in NaCl treatment. As the *HvSAMS1* gene promoter contained RAV motif, the expression of *HvSAMS1* gene might be regulated by other osmotic stresses.

The GUS activity of -301/*GUS* construct was increased in tobacco leaves treated with ethylene. One GCC box located within -276 to -270 bp of the *HvSAMS1* gene promoter. GCC box (AGCCGCC) is found in the promoter regions of many pathogen-responsive genes (Hong et al., 2005) and has the function as ethylene-responsive element. GCC box regulated the transcript of ethylene and played important roles in regulating jasmonate-responsive gene expression (Brown et al., 2003). Deletion analysis of the *HvSAMS1* promoter revealed the elevated GUS activity when the construct included the GCC box. However, non-significant *GUS* expression was also found despite of presence of GARE, RAV, and GCC box. One GARE (-1410 to -1404), four RAV (-994 to -990, -690 to -685, -511 to -507, -473 to -469), and one GCC box (-448 to -442) were observed in -301 upstream sequence of *HvSAMS1* promoter region. The GUS activity controlled by *HvSAMS1* promoter (-1459, -1223, -999, -766 and -545) was not induced by NaCl, ABA and $GA_3$ treatment. This suggested that other *cis*-elements might involve in function of *HvSAMS1* promoter activation under NaCl, ABA and $GA_3$ treatment. In addition, the deletion derivatives of -301 region (ethylene) and -211 region (NaCl, ABA and $GA_3$) increased the GUS expression. These results provided an evidence for the presence of negative regulatory sequences of the *HvSAMS1* promoter at the region between -1459 and -302.

The GUS activity driven by the *HvSAMS1* promoter (-1459, -1223, -999, -766, -545 and -301) was not induced in tobacco leaves 24h after the treatment of each ethionine and spermidine. This result indicated that the exogenous application of ethionine and spermidine was ineffective for the induction. A point mutant *Arabidopsis* plant of *SAMS gene, mto3*, was resistant to ethionine. The *mto3* plants strongly decreased the SAMS activity and SAM concentration (Shen et al., 2002). However, *SAMS* gene transcript was barely detected in barley and tobacco leaves. This can at least partly be explained by the translational and /or post-transcriptional regulation of the *HvSAMS1* gene expression.

Kim et al. (1995) reported that SAMS activity was increased by putrescine treatment for two days after the germination, but L-methionine, spermidine and spermine did not affect on the enzyme activity (Kim et al., 1995). We speculated that the *HvSAMS1* gene was not affected by the regulation of the transcriptional and translational procedures in spermidine treatment.

In conclusion, the *HvSAMS1* promoter was activated locally by growth hormones and abiotic stresses. The same expression patterns in response to the phytohormones and abiotic stresses were observed for constructs containing the -211 to -141 promoter region. Therefore, the analysis of the *HvSAMS1* promoter provided a clue as to the existence of novel *cis*-acting elements, which might be required a transcription factor under the treatment with NaCl, ABA and $GA_3$.

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