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SHORT COMMUNICATION

Temporal and Spatial Expression Patterns of the Gene *AtBSMT1* Encoding a Salicylic Acid Methyltransferase in Arabidopsis Transgenic Plants

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Abstract Expression patterns of *AtBSMT1* encoding an *S*-adenosyl-L-methionine: salicylic acid carboxyl methyltransferase from *Arabidopsis thaliana* were analyzed in *AtBSMT1* promoter::β-glucuronidase (*GUS*) transgenic Arabidopsis. *AtBSMT1* was expressed in flowers and siliques and induced under specific biotic/abiotic stress conditions. The results indicated that the induction of *AtBSMT1* is regulated by plant disease response and jasmonic acid signaling.

Keywords jasmonic acid · plant defense · salicylic acid · salicylic acid methyltransferase

Plants combine vascular and airborne transport of signals to attain a carefully balanced systemic defense reaction to herbivore attack and pathogen infection. For a substance to be considered a translocated signal, it must induce a defensive response, be produced or released at the site of attack, be translocated from the site of the attack to the systemic tissue, and accumulate in the systemic tissue before resistance expression occurs (Heil and Ton, 2008). Specific volatile organic compounds that act as defensive metabolites are considered as translocated signals in plant defense response.

During systemic acquired resistance (SAR), methyl salicylate (MeSA), a volatile secondary metabolite, functions as a critical mobile signal (Park et al., 2007; Sendon et al., 2011). MeSA is synthesized from salicylic acid (SA) by the *S*-adenosyl-L-methionine: salicylic acid carboxyl methyltransferase (SAMT) enzyme (Chen et al., 2003). The SAMT gene, which catalyzes the reaction between SA and the methyl donor, *S*-adenosyl-L-methionine (SAM), to form MeSA, was first identified in *Clarkia breweri* (Ross et al., 1999). Since then, several studies on SAMTs and MeSA emissions have been conducted. A rice SAMT, *OsBSMT1*, overexpressed in Arabidopsis plants was found to generate MeSA-overproducing transgenic plants, demonstrating that induction of the SAMT gene was sufficient to generate an airborne signal resulting in activation of defense responses in neighboring plants (Koo et al., 2007). However it should be noted that the higher rate of conversion of SA to MeSA led to depletion of the free SA pool, thereby increasing the susceptibility of transgenic plants to pathogens. In tomato, *Solanum lycopersicum* SAMT, SlSAMT methylates SA, and constitutive overexpression of the SlSAMT1 gene resulted in greatly increased MeSA emissions from leaves and fruit (Tieman et al., 2010). In rice, OsBSMT1 has both SAMT and benzoic acid (BA) methyltransferase (BAMT) activities. Analyses of *OsBSMT1* expression indicated involvement of the JA and SA signaling pathways and crosstalk between the two pathways during MeSA production (Koo et al., 2007; Zhao et al., 2010).

Induction of SAMT genes can occur following the application of biotic and abiotic stresses (Chen et al., 2003; Kwon et al., 2009; Song et al., 2009). In Arabidopsis, *AtBSMT1* induction was observed following treatment with alamethicin, *Plutella xylostella* herbivory, uprooting, physical wounding, and exposure to methyl jasmonate (MeJA) (Chen et al., 2003). High levels of *AtBSMT1* induction in SA-depleted mutants, in which crosstalk between SA and JA resulted in strong JA signals, supported the idea that *AtBSMT1* gene regulation was regulated by JA signaling (Song et

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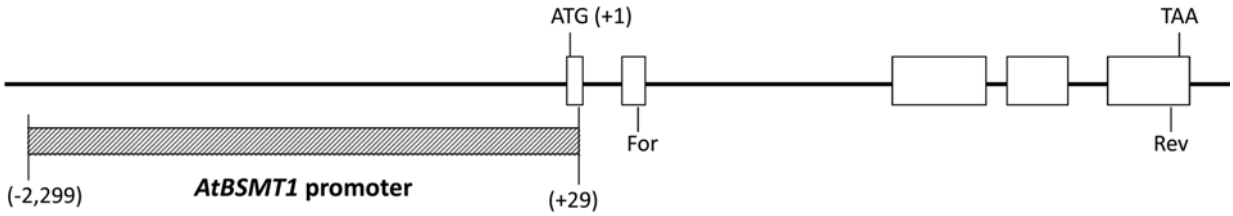

Fig. 1 Gene structure of *AtBSMT1*. The *AtBSMT1* promoter region, −2,299 to +29 bp, where A of ATG is +1 bp, is indicated by the shaded box, while open boxes indicate exons. The For and Rev primer set used for RT-PCR of *AtBSMT1* is also shown.

al., 2009). The expression patterns of the *Atropa belladonna* SAMT gene, *AbSAMT1*, were characterized in *A. belladonna*. *AbSAMT1* was expressed after exogenous SA treatment and 1,2-benzisothioazole-1,1-dioxide (BIT), an active SAR chemical inducer, as well as *Pseudomonas syringae* pv. *tabaci* (*Pst*) infection, physical wounding, and MeJA application (Kwon et al., 2009). Unlike *AtBSMT1*, *AbSAMT1* appears to be under the regulation of two signaling pathways, SA-dependent and JA-dependent responses.

AtBSMT1 was identified by Chen et al. (2003) using a biochemical genomics approach. Among several candidate genes, *At3g11480* was named as *AtBSMT1*, because it showed an expression pattern that perfectly correlated with MeSA emission and the protein encoded exhibited a methylating activity with SA and BA. Under herbivore damage, *AtBSMT1* promoter was strongly expressed around lesions on the leaves.

To further examine the mechanism involved in *AtBSMT1* regulation, expression patterns of *AtBSMT1* were analyzed in *AtBSMT1* transgenic Arabidopsis plants under normal and biotic/abiotic stress conditions. To accomplish this, the *AtBSMT1* promoter region containing 2,299 bp upstream and 26 bp downstream of the translation initiation ATG codon was amplified from *A. thaliana* genomic DNA by polymerase chain reaction (PCR) using 5'-ATGAATTACCCAAACGGAAA-3' and 5'-GGA ATGGTGTTGATGAATCTTG-3' primers (Fig. 1). The product was inserted into a T-blunt vector, and the fragment was digested with *Hin*dIII. The digested fragment was ligated into pBI101 vector to fuse in-frame to the 5' end of the β-glucuronidase (GUS) gene. The construct was then introduced into six-week-old Arabidopsis plants (Columbia background) via *Agrobacterium tumefaciens* GV3101 using the floral dip transformation procedure (Clough and Bent, 1998). *AtBSMT1* promoter::*GUS* transgenic plants were selected by germinating sterilized seeds pretreated with 70% ethanol on MS media containing 40 μg mL^{-1} kanamycin and subsequently grown in soil at 22°C under a 16 h light/8 h dark cycle.

Organ-specific expression of *AtBSMT1* was determined under normal growth conditions using a GUS assay (Fig. 2A). Briefly, one- and two-week old seedlings, leaves, flowers, and siliques were incubated overnight in GUS-staining solution containing 0.1 M sodium phosphate at pH 7.0, 0.1% Triton X-100, 0.5 mM $K_3(Fe[CN]_6)$, 0.5 mM $K_4(Fe[CN]_6)$, and X-Gluc (5-bromo-4-chloro-3-indolyl β-glucuronide) (MB Cell, USA) at 37°C. The samples were then placed in 70% ethanol to extract the chlorophyll. A blue color in the samples indicated the presence of GUS activity, which served as a marker for the presence of *AtBSMT1* promoter activity. *AtBSMT1* was expressed in flowers and siliques, and *AtBSMT1* promoter activity was localized to the sepals, stamen, and stigma. Within the silique, its activity was localized in the region of the receptacle and stigma. However, *AtBSMT1* was not expressed in leaves, roots, and seedlings. Reverse transcriptase (RT)-PCR conducted using the For (5'-CCAACAGTTACTCCGCCAAT-3') and Rev (5'-GTTGGCATGT TGAGTCACATGGT-3') primer set with an expected product size of 980 bp (Fig. 1) to determine the expression levels of *AtBSMT1* in different organs confirmed the results of the GUS assay (Fig. 2B).

Different forms of biotic and abiotic treatments were applied to the plants. Infection of 18-day old transgenic plants through infiltration with *P. syringae* pv. *maculicola* strains DG3 and DG6 was conducted as described by Greenberg et al. (2000), and leaf samples were collected after 12 and 24 h. Wounding was performed by applying force to the surface of the leaves using gritted-surface scissors. Leaf sampling was conducted after 2 and 6 h. MeSA and MeJA treatments were applied by spraying plants with solutions containing 100 μM of the compounds until all leaves were wet. The plants were then placed in covered containers until leaf samples were harvested at 12 and 24 h. Finally, the expression patterns were checked using a GUS assay and RT-PCR.

Fig. 3 shows the expression patterns of *AtBSMT1* after application of different treatments. The results revealed that *AtBSMT1* was induced upon pathogen infection of the leaves with DG3 and DG6 strains of *P. syringae*. Expression was observed at 12 h after pathogen infection, but was higher after 24 h. *AtBSMT1* was also induced after physical wounding, being highly upregulated two h after wounding but declined rapidly after 6 h. Finally, *AtBSMT1* was induced by MeJA; however, no difference in the expression levels was observed at 12 and 24 h after treatment. MeSA application did not induce *AtBSMT1*.

Results of the present study indicated that *AtBSMT1* induction is regulated by two mechanisms, pathogen defense response and JA signaling. During pathogen defense response, SA accumulates and its metabolites are synthesized (Song et al., 2009). Induction

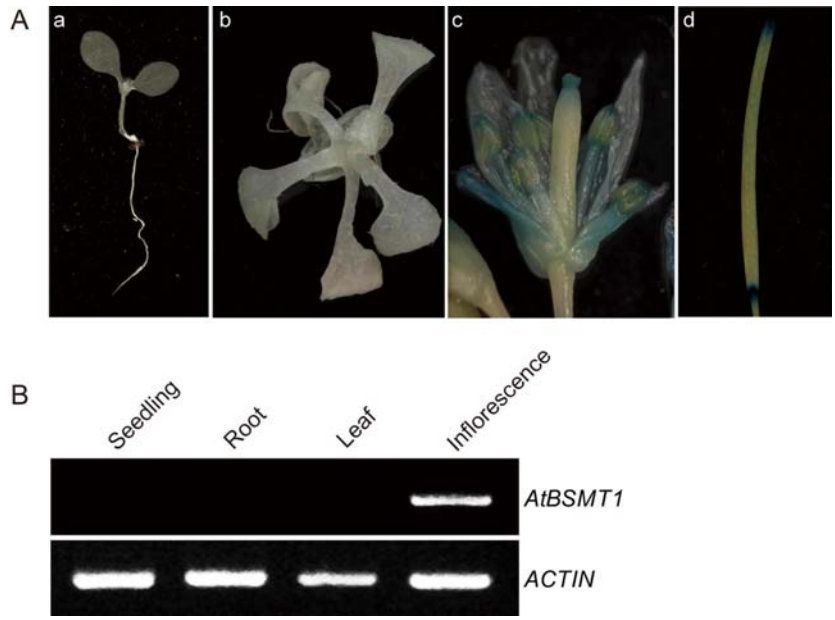

Fig. 2 Expression pattern analysis of *AtBSMT1* in different organs. A. GUS staining of (a) one-week old seedlings, (b) two-week old plants, (c) flowers, and (d) silique in *AtBSMT1* promoter::*GUS* transgenic plants. B. RT-PCR analysis of *AtBSMT1* transcript levels in seedlings, roots, leaves, and flowers. Actin was used as a control marker.

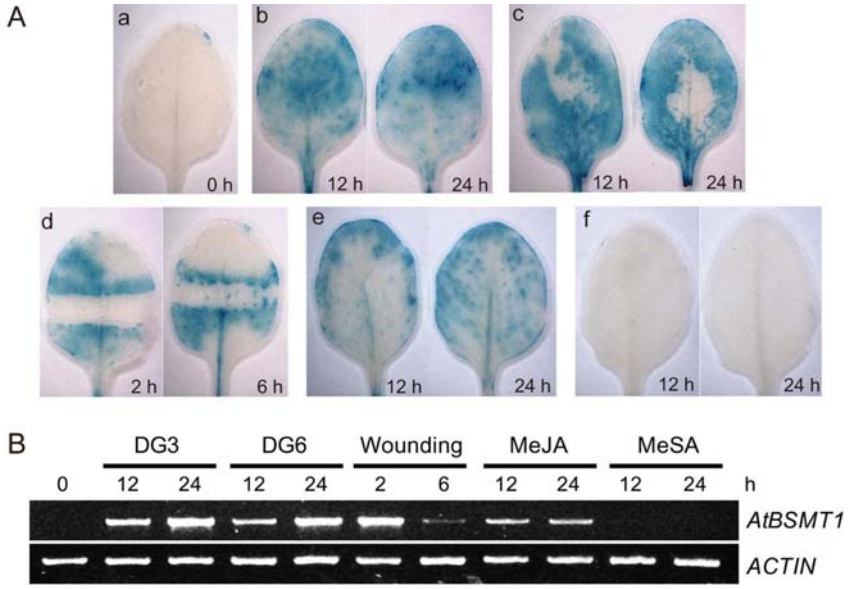

Fig. 3 Expression pattern analysis of *AtBSMT1* after biotic and abiotic treatments. A. GUS staining of (a) untreated leaves, (b) DG3-infected leaves, (c) DG6-infected leaves, (d) wounded leaves, and leaves treated with (e) MeJA and (f) MeSA in *AtBSMT1* promoter::*GUS* transgenic plants. B. RT-PCR analysis of *AtBSMT1* transcript levels in untreated leaves, DG3-infected leaves, DG6-infected leaves, wounded leaves, and leaves treated with MeJA and MeSA. Actin was used as a control marker.

of *AtBSMT1* by *P. syringae* infection leads to the production of MeSA and continuous MeSA synthesis eventually results in decreased SA levels. SA and JA crosstalk or exert an antagonistic effect on each other. While SA is undergoing negative regulation, JA becomes positively regulated, leading to activation of the JA-responsive regulatory genes. During wounding or when MeJA is applied, *AtBSMT1* is induced and JA-responsive genes are activated while SA-responsive genes are repressed. Koo et al. (2007) discussed events occurring during SA and JA crosstalk. In their scenario, SA defense response is triggered during pathogen infection by certain regulatory genes or transcription factors, such as non-expressor of pathogenesis-related genes 1 (NPR1) and

WRKY70. NPR1 represses *LOX2*, a JA biosynthetic gene, which leads to the repression of JA synthesis and JA defense response. Conversely, JA/MeJA represses WRKY70 and its responsive genes and induces *SAMT*, resulting in reduced SA levels and suppression of SA signaling. Koo et al. (2007) suggested that induction of the SAMT gene is a crucial switch between SA and JA signaling pathways, which is supported by the results of our study.

Due to the significant role of *AtBSMT1*, its possible regulators are currently being investigated by isolating and characterizing mutants exhibiting loss-of-function or gain-of-function from an activation tagging line of *AtBSMT1* promoter::*GUS* transgenic plants. Functional analysis of the regulator(s) is expected to lead to successful elucidation of the genetic pathway of *AtBSMT1*.

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