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Original Article

GUS Expression Driven by Promoter of AtSAGT1 Gene Encoding a Salicylic Acid Glucosyltransferase 1 in Arabidopsis Plants

Pamella Marie Sendon, Jong-Beum Park, Soon-Ki Park, Jong Tae Song*
School of Applied Biosciences, Kyungpook National University, Daegu 702-701, Korea

Abstract

AtSAGT1 encodes a salicylic acid (SA) glucosyltransferase enzyme that catalyzes the formation of SA glucoside and SA glucose ester. Here, the *AtSAGT1* gene expression patterns were determined in *AtSAGT1* promoter::*GUS* transgenic Arabidopsis plants. As a result, the factors regulating the induction of *AtSAGT1* were identified as pathogen defense response, wound response, exogenous application of SA, and jasmonic acid treatment.

Keywords : Arabidopsis, *AtSAGT1*, GUS, salicylic acid, salicylic acid glucosyltransferase

Introduction

Salicylic acid (SA) is an endogenous signaling molecule in plants that mediates defense responses against pathogens (Chen et al. 2009; Lu 2009; Umemura et al. 2009; Rivas-San Vicente and Plasencia 2011; Sendon et al. 2011). Increased production of SA leading to the accumulation of pathogenesis-related (PR) proteins is an essential process in both local and systemic acquired resistance (Loake and Grant 2007). Local acquired resistance (LAR) occurs at the site of infection and immediate surroundings, while systemic acquired resistance (SAR) involves the long distance transport of SA from the tissue expressing the hypersensitive response to other parts of the plant (Lee et al. 1995; Metraux 2002).

SA is synthesized through two distinct pathways: the phenylpropanoid and the isochorismate pathways. The phenylpropanoid pathway, which occurs in the cytoplasm, involves the conversion of phenylalanine to SA, while the isochorismate pathway, which occurs in the chloroplast, involves the synthesis of SA from chorismate (Ryals et al. 1996; Durrant and Dong 2004; Rivas-San Vicente and Plasencia 2011, Sendon et al. 2011). SA is then metabolized through glucosylation and/or methylation. Glucosylation at the hydroxyl group of SA results in SA glucoside [SA 2-*O*-β-D-glucose] (SAG) formation as a major metabolite, while glucose conjugation at the carboxyl group results in the formation of SA glucose ester (SGE) as a minor metabolite (Rivas-San Vicente and Plasencia 2011). Methylation, on the other hand, results in the formation of methyl salicylate (MeSA) (Chen et al. 2003; Song et al. 2009).

SA glucosyltransferase (SAGT) enzymes catalyze the conversion of SA to SAG and SGE. Arabidopsis contains two SAGT enzymes: UGT74F1 also known as AtSAGT2, which leads to the formation of SAG, and UGT74F2 also known as AtSAGT1, which results in the formation of both SAG and SGE (Dean and Delaney 2008; Song et al. 2008). UGT74F1/AtSAGT2 is encoded by *At2g43840* and UGT74F2/AtSAGT1 is encoded by *At2g43820* (Dean and Delaney 2008). SAGT plays a vital role in plant defense response, and its induction and regulation have already been investigated in several studies. Tobacco mosaic virus (TMV) inoculation into tobacco leaves resulted in the endogenous synthesis of SA and rapid conversion of SA to SAG, suggesting the occurrence of SAGT activity (Enyedi et al. 1992). An increase in SAGT activity was found to correspond with SA accumulation and SAG formation (Lee et al. 1995). In rice, a putative OsSGT1 gene was cloned and the results showed that OsSGT1 was involved in SAG production and was a key mediator in the development of chemically induced resistance (Umemura et al. 2009). Song (2006) isolated the AtSAGT1 gene (formerly named AtSGT1) in Arabidopsis and obtained a recombinant AtSAGT1 protein exhibiting significant activity toward SA and benzoic acid. The results also showed that the AtSAGT1 gene was induced by exogenous SA and *Pseudomonas syringae* infection. In addition, expression patterns of *AtSAGT1* in Arabidopsis plants with reduced SA signaling were identified (Song et al. 2009). *AtSAGT1* induction after pathogen infection was reduced in the following mutants with defense signaling defects: *pad4,*

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*Corresponding Author: Jong-Tae Song, Tel. 82-53-950-7753, Fax. 82-53-958-6880, Email. jtsong68@knu.ac.kr

npr1, nahG, and sid2, while AtSAGT1 was strongly expressed in constitutively defensive mutants, *agd2 and acd6.*

In this study, several factors affecting *AtSAGT1* induction were determined. The expression patterns of *AtSAGT1* were analyzed using the β-glucuronidase (GUS) activity in Arabidopsis *AtSAGT1* promoter::*GUS* transgenic plants. The region of the *AtSAGT1* promoter was amplified by a polymerase chain reaction (PCR) using *A. thaliana* genomic DNA with the following set of primers, 5'-TCGTGTTTTGATGACGCAAT-3' and 5'-GGATCCGTCGAGCCAGTTAATGCAGA-3'. The promoter region consisted of 1,174 bp upstream and 861 bp downstream of the translation initiation ATG codon (Figure 1). The product was inserted into a T-blunt vector (Solgent, Korea) and digested with *Bam*HI. The fragment was then ligated into a pBI101 vector (Clontech) to fuse the in-frame to the 5'end of the GUS gene. The resulting construct was introduced into six-week-old Arabidopsis plants (Col background) by means of *Agrobacterium tumefaciens* GV3101 using a floral dip transformation procedure (Clough and Bent 1998). The resulting *AtSAGT1* promoter::*GUS* transgenic plants were then screened by germinating sterilized seeds pretreated with 70% ethanol on MS media containing 40 μg mL^{-1} kanamycin and grown in soil at 22℃ under a 16 h light/8 h dark cycle.

The expression of *AtSAGT1* was determined under normal and treated conditions using a GUS assay. Leaves from the 20-day-old transgenic plants were incubated in a 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-3-3-indolyl β-glucuronide) (MB Cell, USA) at 37℃ overnight. The samples were then placed in 70% ethanol to extract the chlorophyll. Development of a blue color in the samples indicated the occurrence of GUS activity, which serves as a marker for the presence of *AtSAGT1* promoter activity.

Different forms of treatment were applied to the plants. For pathogen infection, the 20-day-old transgenic plants were infiltrated with *P. syringae* pv. *maculicola* strains DG3 (virulent) and DG6 (avirulent) (Greenberg et al. 2000). Leaf samples were then collected after 12 h and 24 h. Wounding was performed by applying force to the surface of the leaves using gritted-surface scissors, and leaf samples then collected after 2 h and 6 h. The application of methyl jasmonate (MeJA) and methyl salicylate (MeSA) was performed by spraying the plants with 100 μM of the said compounds until all the leaves were wet. The plants were then placed in covered containers and leaf samples collected after 12 h and 24 h. A GUS assay and reverse transcriptase (RT)-PCR were performed to check the *AtSAGT1* expression patterns. The RT-PCR was conducted using the For 5'-TTCTCTGTTTCTGGCTCTTATCC-3' and Rev 5'-TAAT-GCAGAAGGAATCATCTTTCG-3'primers, with an expected product size of 542 bp (Figure 1).

Figure 2 shows the expression patterns of *AtSAGT1* after applying the different treatments. GUS expression was observed upon infiltration of the leaves with the DG3 and DG6 strains of *P. syringae.* Plus, the RT-PCR analysis showed that the *AtSAGT1* gene was expressed at both 12 h and 24 h after pathogen infection. GUS expression was also observed after exogenous application of MeJA and MeSA, and the RT-PCR showed *AtSAGT1* gene expression at 12 h and 24 h after treatment. *AtSAGT1* was also induced at 2 h and 6 h after wounding.

SA plays a significant role in disease resistance. However, SA is considered phytotoxic in concentrations greater than 0.1 mM (Lee et al. 1995). SA metabolism and the formation of SA conjugates can function in SA detoxification and as a slow-release storage form of SA to maintain SAR over a prolonged period of time (Lee et al. 1995). SAGT catalyzes the formation of SAG and SGE. In Arabidopsis, SAGT activity is demonstrated by the AtSAGT1 protein. Several studies have shown that SAGT is induced by infection. During pathogen infection, SA is

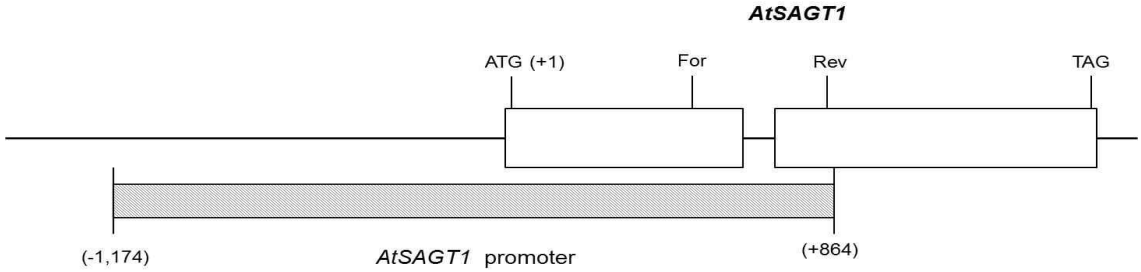

Figure 1. Gene structure of *AtSAGT1*. *AtSAGT1* promoter region, indicated by shaded box, -1,174 bp to +864 bp, where A of ATG is +1 bp, was fused to GUS gene. Two exons of *AtSAGT1* are represented by white boxes. *AtSAGT1* RT-PCR product (542 bp) was amplified using For and Rev primer set.

produced locally and systemically (Summermatter et al. 1995; Metraux 2002; Lu 2009). TMV inoculation in tobacco leaves led to the endogenous synthesis of SA, which was rapidly converted to SAG by the tobacco SAGT gene (Enyedi et al. 1992; Malamy et al. 1992). *P. syringae* infection in Arabidopsis resulted in *AtSAGT1* induction (Song et al. 2006). Here, an increased *AtSAGT1* transcript level was observed and correlated with SA accumulation. Thus, the present results confirmed that *AtSAGT1* is induced by *P. syringae* infection. A high GUS expression was also observed at both 12 h and 24 h after DG3 and DG6 infection (Figure 2A) corresponding to high *AtSAGT1* transcript levels (Figure 2B).

During wounding, JA levels increase locally in response to tissue damage (Baldwin et al. 1997). In a review by Heil and Ton (2008), wound response involves the synthesis of JA, its precursors, and derivatives. Linolenic acid is converted into JA through multiple steps and several enzymes. JA derivatives, also known as jasmonates, are then subsequently transported to elicit a systemic response. Due to antagonistic interaction, JA negatively regulates the SA signaling pathway (Loake and Grant 2007). JA induces SA methyltransferase, AtBSMT1, to convert SA to MeSA, resulting in SA reduction (Koo et al. 2007). The results of this study also showed that JA induces AtSAGT1, thereby converting SA to SAG and SGE to aid in SA reduction. Plus, high *AtSAGT1* induction was observed 6 h after wounding (Figure 2B).

AtSAGT1 induction also resulted from the exogenous application of MeJA. The mode of action following MeJA treatment is similar to that after wounding. JA levels increase after MeJA application. JA signaling is activated and SA signaling is suppressed. *AtSAGT1* is therefore induced to decrease the levels of SA. Here, the RT-PCR results showed high transcript levels of *AtSAGT1* at 12 h and 24 h after MeJA treatment (Figure 2B). GUS expression was also high at 12 h, yet decreased at 24 h (Figure 2A). Although high levels of *AtSAGT1* transcripts may still have been present at 24 h, the SA levels had already declined, slowing the enzymatic activity of ATSAGT1.

Finally, the application of MeSA also induced the expression of the *AtSAGT1* gene. High GUS expression and *AtSAGT1* transcript levels were observed at 12 h and 24 h after MeSA

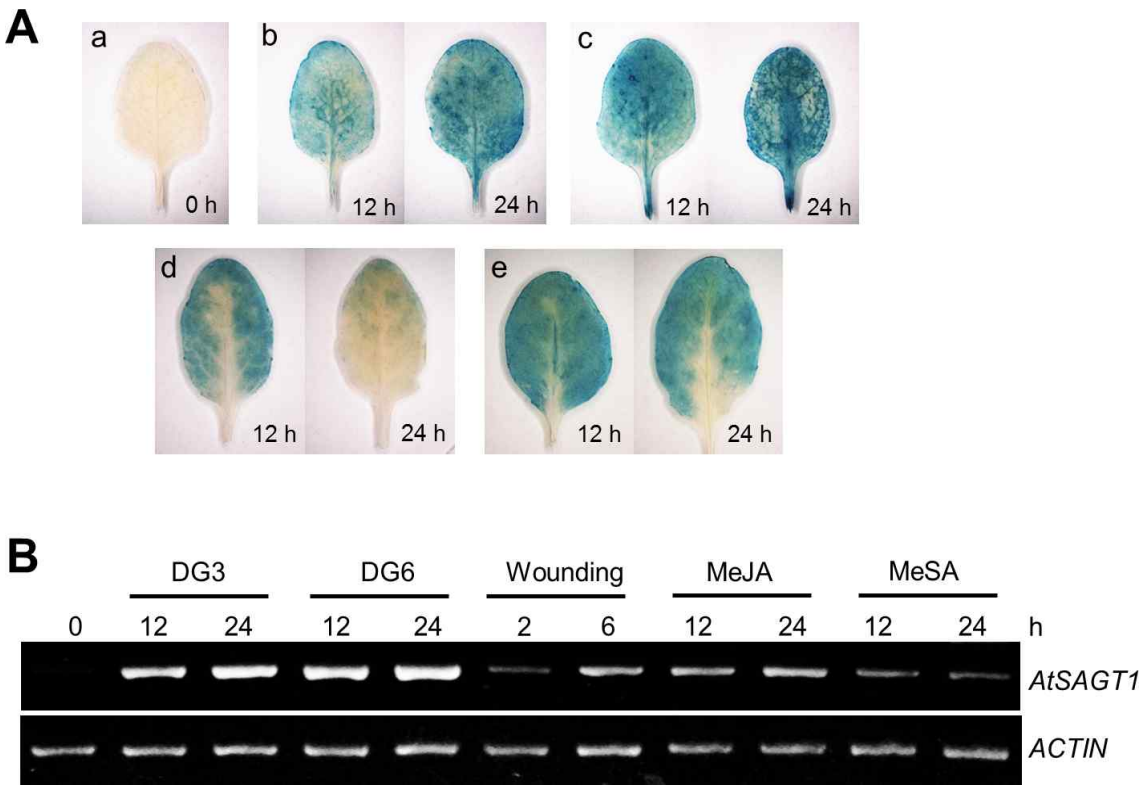

Figure 2. Analysis of *AtSAGT1* expression. A. GUS activity assay in *AtSAGT1* promoter::*GUS* transgenic plants. GUS staining was performed using untreated leaf (a), DG3-infected leaves (b), DG6-infected leaves (c), MeJA application (d), and MeSA application (e). B. RT-PCR analysis of *AtSAGT1*. Total RNAs were isolated from untreated leaves, DG3-infected leaves, DG6-infected leaves, wounded leaves, MeJA treated leaves, and MeSA treated leaves. Actin was used as control marker.

treatment. MeSA is a mobile signal involved in SAR (Park et al. 2007). MeSA is converted to SA by methyl salicylate esterase Salicylic Acid Binding Protein 2 (SABP2) (Forouhar et al. 2005). An increase in the MeSA concentration results in increased levels of SA. SA will then be synthesized into SAG and SGE by AtSAGT1 to maintain an SA level that is non-toxic to plants.

Complex molecular mechanisms are involved in SA and its role in both local and systemic acquired resistance. One vital part of the multifaceted regulation is SA metabolism involving SA glucosyltransferases and SA methyltransferases. This study identified the conditions when *AtSAGT1*, encoding an SA glucosyltransferase, induction occurs, which include pathogen defense response, wounding, JA application, and SA treatment. However, since the current results only represent part of the larger picture, further studies are needed to fully understand the complexity and significance of SA metabolism.

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