

# Overexpression of the 3'(2'),5'-Bisphosphate Nucleotidase Gene *AtAHL* Confers Enhanced Resistance to *Pectobacterium carotovorum* in *Arabidopsis*

Hyon Jin Park · **Yang Do Choi** · Sang Ik Song · Hawk-Bin Kwon · Nam Iee Oh · **Jong-Joo Cheong**

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**Abstract** The *Arabidopsis AtAHL* gene encodes a 3'(2'),5'-bisphosphate nucleotidase that catalyzes the conversion of adenosine 3',5'-bisphosphate (PAP) into adenosine monophosphate and inorganic phosphate. We have generated transgenic *Arabidopsis* overexpressing this gene under control of the cauliflower mosaic virus 35S (CaMV 35S) promoter. Transgenic lines integrating a single copy of the insert DNA and constitutively expressing the *AtAHL* gene were selected. The transgenic lines of *Arabidopsis* plants exhibited enhanced resistance to *Pectobacterium carotovorum* subsp. *carotovorum*. In general, plant defense responses and sulfur metabolism are linked through jasmonic acid signaling. The expression of sulfur-related defense genes is known to be induced via a jasmonate-mediated signaling pathway. In this work, we observed that the expression of *AtAHL* was also induced by jasmonate treatment in *Arabidopsis*. Our data suggest that PAP catabolic activity enhanced by the jasmonate signaling pathway

contributes to the rapid flux of the sulfur activation pathway, accelerates the incorporation of activated sulfur into sulfur-containing defense molecules such as defensins, thionins, and glucosinolates, and thereby increases defense resistance in plants.

**Keywords** *Arabidopsis* · *AtAHL* · 3'(2'),5'-bisphosphate nucleotidase · disease resistance · *Pectobacterium carotovorum*

## Introduction

Sulfur is an essential element in living organisms. In particular, the sulfur-containing amino acids, cysteine and methionine, are found in almost all cellular proteins as building units (Leyh, 1993; Droux, 2004). Moreover, sulfur exerts critical roles in defense mechanisms in plants. Elemental sulfur has been used as a fungicide for more than 100 years (Cooper and Williams, 2004), and many sulfur-containing compounds, including defensins, thionins, and glucosinolates, have been linked to plant defense mechanisms against a broad spectrum of pathogens (Rausch and Wachter, 2005; Kruse et al., 2007).

To utilize sulfur in the inorganic state (sulfate), it must be converted to a reduced form (sulfide). In plants, sulfate is activated via coupling with ATP to form adenosine 5'-phosphosulfate (APS) by ATP sulfurylase, and reduced to sulfite by APS reductase (Kopriva and Koprivova, 2004; Kopriva, 2006; Takahashi et al., 2011). In a separate branch of the pathway, APS kinase phosphorylates APS to 3'-phosphoadenosine 5'-phosphosulfate (PAPS). PAPS is reduced by PAPS reductase to sulfite, which is further reduced to sulfide by sulfide reductase. A putative PAPS reductase was discovered in moss as a novel form of APS reductase without an iron-sulfur cluster (Kopriva et al., 2002; 2007).

H. J. Park  
Present address: R&D Center, CELLTRION Inc., Incheon 406-840, Republic of Korea

H. J. Park · Y. D. Choi  
Department of Agricultural Biotechnology, Seoul National University, Seoul 151-921, Republic of Korea

S. I. Song  
Division of Bioscience and Bioinformatics, Myongji University, Yongin 449-728, Republic of Korea

H.-B. Kwon  
Division of Biomedical Sciences, Sunmoon University, Asan 336-708, Republic of Korea

N. I. Oh · J.-J. Cheong (✉)  
**Center for Food and Bioconvergence**, Seoul National University, Seoul 151-921, Republic of Korea  
E-mail: cheongjj@snu.ac.kr

Together with APS, PAPS serves as the source of a variety of sulfate transfer reactions (Klaassen and Boles, 1997). In particular, PAPS is required for the biosynthesis of glucosinolates, a group of defense compounds (Rausch and Wachter, 2005; Falk et al., 2007). Glucosinolates have a sulfonated oxime, a thioglucose moiety, and a side chain derived from aliphatic and aromatic amino acids; their metabolites in plant cells mediate resistance to microbial pathogens (Halkier and Gershenzon, 2006; Sønderby et al., 2010).

In the sulfur transfer reactions utilizing PAPS, 3'-phosphoadenosine 5'-phosphate (PAP) is generated as a by-product. The PAP is then hydrolyzed to AMP and inorganic phosphate by a PAP-specific phosphatase, 3'(2'),5'-bisphosphate nucleotidase. The proteins encoded by yeast *HAL2* (Murguía et al., 1996), *Arabidopsis SAL1* (Quintero et al., 1996) and *Arabidopsis AtAHL* (Cheong and Kwon, 1999; Gil-Mascarell et al., 1999) have such PAP nucleotidase activity. These enzymes may contribute to a rapid sulfur flux through the sulfate activation pathway by accelerating the PAPS-utilizing reactions. Supporting this postulation, inhibition of the yeast *HAL2* enzyme by  $\text{Li}^+$  or  $\text{Na}^+$  treatment raises the intracellular concentrations of PAP and PAPS, resulting in inhibition of sulfate assimilation (Murguía et al., 1996).

Attempts have been made to generate transgenic plants with enhanced formation of sulfur-containing compounds and thereby enhancing defense responses in plants. However, direct reinforcement of the sulfate activation pathway may be harmful to plants, because PAP is toxic to cells when accumulated at high concentrations (Yang et al., 1994; Peng and Verma, 1995; Dichtl et al., 1997; Klaassen and Boles, 1997).

In the present study, we generated transgenic *Arabidopsis* that overexpressed the *AtAHL* gene, and observed that the transgenic plants exhibited enhanced resistance to the non-specific bacterial soft-rot pathogen *Pectobacterium carotovorum* subsp. *carotovorum* (formerly *Erwinia carotovora* subsp. *carotovora*). The PAP nucleotidase activity exerted by the *AtAHL* enzyme could increase the rate of sulfur flux in the sulfate activation pathway, and accelerate the detoxification of PAP accumulated in the infected cells of the transgenic *Arabidopsis*. In addition, we observed that the *AtAHL* gene was induced by jasmonate, likely similar to other genes involved in the sulfate activation pathway.

## Materials and Methods

**Growth conditions of *Arabidopsis*.** The *Arabidopsis thaliana* Columbia (Col-0) ecotype was used throughout the experiments. Seeds of *Arabidopsis* were stored at 4°C until use. The seed surface was sterilized by soaking in 70% (v/v) ethanol for 15 min and in 100% ethanol for 5 min. The seeds were put on an autoclaved filter paper and dried in a laminar flow clean bench. After sowing on solid half-strength Murashige and Skoog (MS) agar medium (Duchefa, Netherlands), seeds were vernalized at 4°C for 4 days. Then the plates were incubated in a growth

chamber, keeping the conditions of 60% relative humidity at 22°C under a 16-h photoperiod at 500 mol m<sup>-2</sup> · s<sup>-1</sup> photon flux density. After 2 weeks of incubation, seedlings were transferred to soil and grown for 3 weeks under the same conditions as in the growth chamber.

For jasmonate treatment, 10 mL methyl jasmonate (MeJA; 100 μM in 100% methanol) was poured into the plates in which 3-week-old seedlings were growing. During the treatment, the plates were sealed with Parafilm, and whole plantlets were harvested 1, 3, 6, 12, and 24 h after the treatment.

**Transgenic *AtAHL* overexpression plants.** To generate transgenic plants integrating the gene, the full-length *AtAHL* cDNA (EST clone 36E7) obtained from The Arabidopsis Information Resource (TAIR) was inserted into the pBI1111L at *Xba*I and *Xho*I sites in sense orientation, downstream of the cauliflower mosaic virus 35S (CaMV 35S) promoter. Thus, the 35S:AtAHL construct constituted, in order, RB (right border)-Pnos-*NptII*-Tnos-P35S-*AtAHL*-Tnos-LB (left border). The DNA construct was transformed into 4-week-old *Arabidopsis* using the *Agrobacterium*-mediated floral dip transformation procedure (Clough and Bent, 1998). Transformed plants were grown further in a growth chamber until they produced seeds. Transgenic plants were then selected by screening for seeds that germinated on kanamycin (30 μg mL<sup>-1</sup>)-containing solid MS medium. The *AtAHL* knockout mutant, Salk\_055685, was obtained from The Salk Institute (<http://signal.salk.edu>).

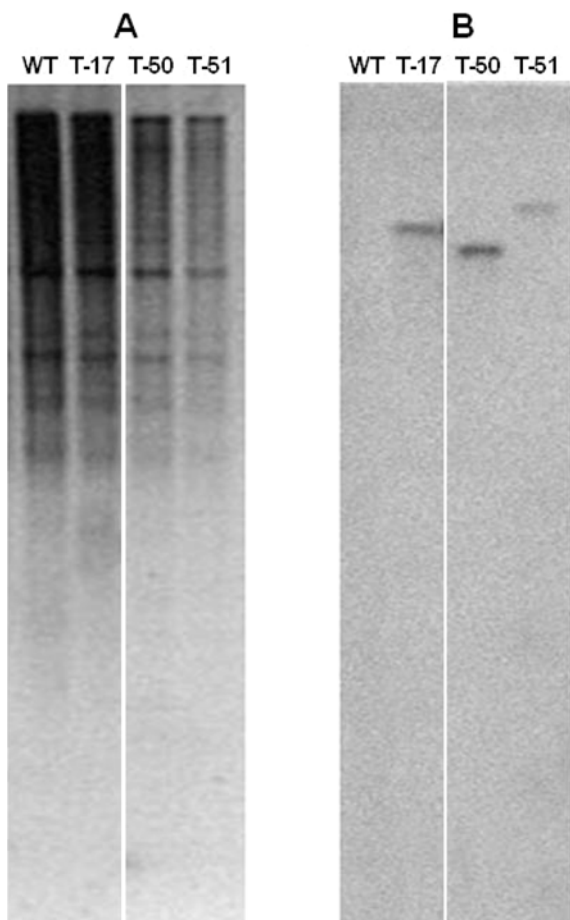
**Blot analyses.** For genomic Southern blot analysis, 5 μg of genomic DNA was digested with a restriction enzyme, separated on 0.8% agarose gels, and transferred to nylon membranes. The blots were then probed with *NPTII* cDNA integrated in transgenic plants as a selection marker.

Northern blot analysis was performed using total RNA extracted from frozen samples with phenol/SDS/LiCl (Carpenter and Simon, 1998). Total RNA (5 μg) was separated on 1.3% agarose formaldehyde gels and transferred to GeneScreen Plus hybridization transfer membranes (Perkin Elmer, USA). The blots were then probed with EST clones obtained from TAIR.

**Pathogen resistance test.** A virulent strain of *P. carotovorum* subsp. *carotovorum* was retrieved from the storage tube and cultured overnight at 28°C in Luria-Bertani medium. Bacteria were harvested by centrifugation (10 min at 5,000 rpm) and then resuspended in 10 mM MgCl<sub>2</sub> (approximately 1 mL) to 10<sup>6</sup> CFU mL<sup>-1</sup>. The bacterial suspension was sprayed on plants. The percentage of dead leaves showing spreading necrosis symptoms was recorded 6 days after the inoculation. Alternatively, a 2-μL droplet of the bacterial suspension was spotted on *Arabidopsis* leaves, and the diameter of necrotic lesions on the leaves formed 6 days after the drop inoculation was recorded.

## Results

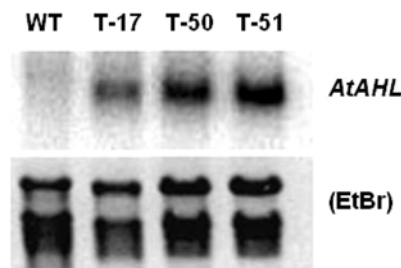
**Transgenic *Arabidopsis* overexpressing *AtAHL*.** Transgenic *Arabidopsis* plants in which *AtAHL* expression is constitutively



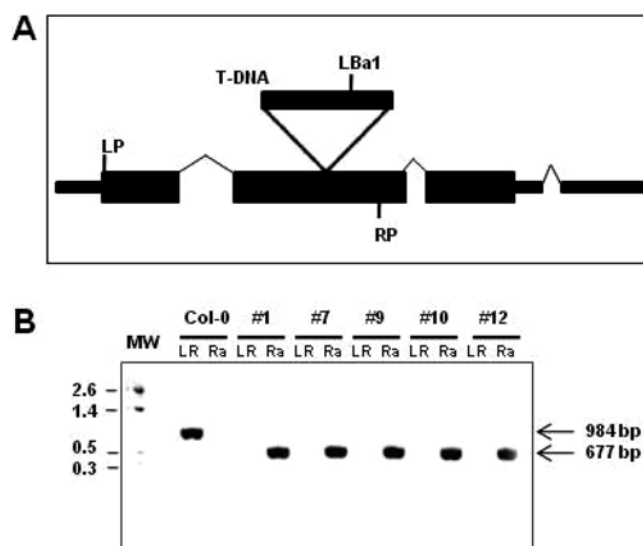
**Fig. 1** Genomic Southern blot analysis of the 35S:*AtAHL* transgenic *Arabidopsis*. Genomic DNA was digested with *Eco*RI. Samples were electrophoresed on 0.8% agarose gel (A), and then the blot was hybridized with a random primed *NPTII* cDNA fragment (B). *AtAHL*-overexpressing transgenic plant lines, T-17, T-50, and T-51, were identified to integrate a single copy of the transgene. WT denotes non-transformed wild-type plants. A region of lines showing multiple bands was excised from the blot for clarification, leaving a gap in the gel between T17 and T-50 transgenic lines.

driven by the CaMV35S promoter were generated by transforming a fused 35S:*AtAHL* DNA construct. T<sub>3</sub> lines of 35S:*AtAHL*-integrating transgenic plants were analyzed with Southern blot analysis to estimate the copy number of the integrated *AtAHL* gene. Genomic DNA was extracted from plants, and 2 μg of genomic DNA was digested with *Eco*RI. The *NPTII* cDNA was used as a hybridization probe. In the genomic Southern blotting, three transgenic lines, T-17, T-50, and T-51, were identified as integrating a single copy of the transformed 35S:*AtAHL* (Fig. 1), while T-24, T-26, and T-33 had multiple copies (data not shown).

Leaf samples were taken from 4-week-old transgenic and non-transformed control plants, and subjected to Northern blot analysis using a random-primed full-length (1,122-bp-long) fragment prepared from *AtAHL* cDNA. The Northern blotting showed that the



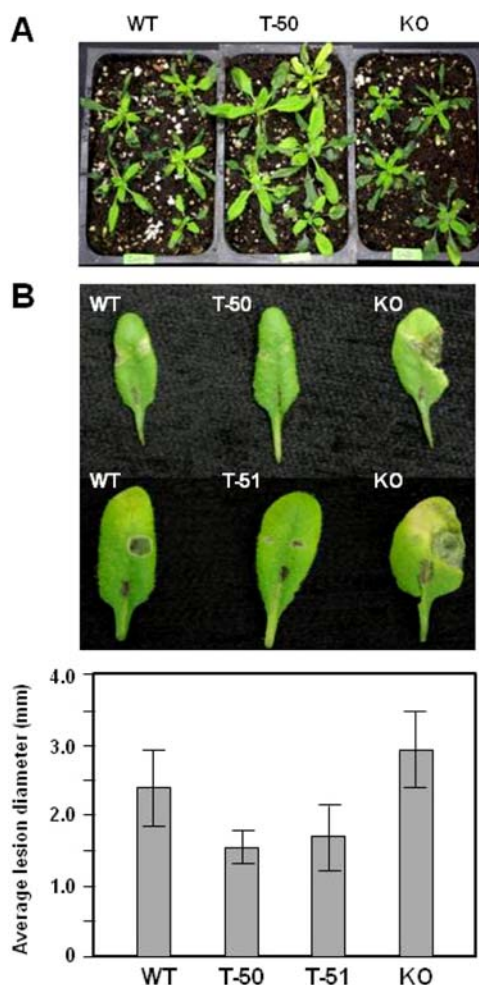
**Fig. 2** Northern blot analysis of the 35S:*AtAHL* *Arabidopsis*. Total RNA was isolated from rosette leaves of wild-type (WT) and transgenic plants (T-17, T-50, and T-51). Five micrograms of total RNA was electrophoresed on 1.3% formaldehyde agarose gel and blotted onto nylon membranes. The blots were hybridized with the full-length (1,122-bp-long) fragment prepared from *AtAHL* cDNA. Loading of equal amounts of RNA was confirmed by ethidium bromide (EtBr) staining of the gel.



**Fig. 3** Polymerase chain reaction (PCR)-based genotyping of the *AtAHL*-knock mutant, salk\_055685. (A) Schematic presentation of T-DNA insertion. The T-DNA is inserted in the second exon of At5g54390 (*AtAHL*). Positions of the primers were indicated; right border genomic primer (RP), left border genomic primer (LP), and insert DNA primer (LBa1). (B) Agarose gel electrophoresis. In the five sub-lines of T-DNA-integrated mutant, 677-bp PCR products were produced between the primers RP and LBa1 (Ra). In the case of the wild type, 984-bp PCR products were made between LP and RP (LR).

*AtAHL* gene was stably overexpressed in the three selected lines, integrating a single copy of the transformed gene and yielding approximately 1.1-kbp-long transcripts (Fig. 2).

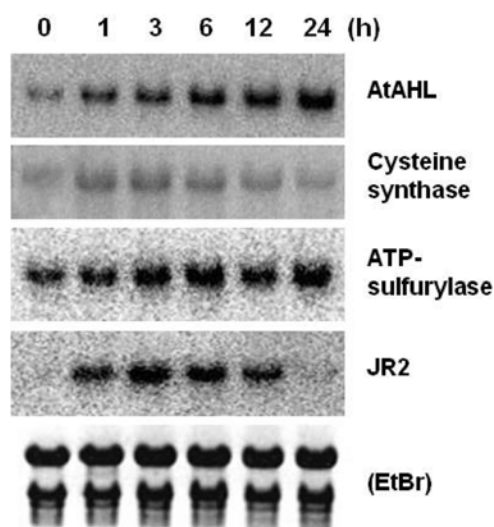
***AtAHL* knockout mutant.** The *AtAHL* knockout mutant, Salk\_055685, was obtained from The Salk Institute (<http://signal.salk.edu>). In this mutant, a T-DNA is inserted into the second exon of At5g54390 (Fig. 3A). Seeds of the T<sub>3</sub> generation were germinated in MS-kanamycin medium, and then genomic DNAs were extracted to select homozygotes using polymerase chain reaction (PCR)-based genotyping. In the T-DNA-integrated



**Fig. 4** Disease resistance of the 35S:*AtAHL* *Arabidopsis*. Five-week-old wild-type (WT), transgenic (T-50, and T-51) and *atahl* knockout (KO) plants were inoculated with (A) spraying or (B) spotting of  $1.0 \times 10^6$  CFU  $\text{mL}^{-1}$  *P. carotovorum*. Images represent results from six independent experiments. The graph in b shows average diameters (and error ranges) of necrotic lesions formed on leaves of 5-week-old *Arabidopsis* plants 6 days after drop inoculation with *P. carotovorum*.

mutant, 677-bp PCR products were produced between the right border genomic primer (RP) and insert DNA primer (LBA1) designed from the *AtAHL* sequence (Fig. 3B). In the case of the wild type, 984-bp PCR products were made between the left border genomic primer (LP) and the RP. Nucleotide sequences of the primers used were LP (5'-TAGAAACGGAGATTGACACGG-3'), RP (5'-AAACCGGATCATCAACTGAAG-3'), and LBA1 (5'-TGGTTCACGTAGTGGGCCATCG-3'). From this experiment, five lines were identified as homozygotes and used in further experiments.

**Resistance to bacterial infection.** Wild-type and transgenic plants were inoculated with  $1.0 \times 10^6$  CFU  $\text{mL}^{-1}$  *P. carotovorum*. A resistance response was observed on leaves of transgenic plants 6 days after infection by both spraying (Fig. 4A) and spotting



**Fig. 5** Jasmonate induction of *AtAHL*. Wild-type *Arabidopsis* plants were treated with 50  $\mu\text{M}$  MeJA, and total RNA were collected at the indicated times. The blots were probed with EST clones obtained from TAIR: cysteine synthase (At3g61440), ATP-sulfurylase (At4g14680), and JR2 (*Jasmonate Responsive 2*) genes. To confirm equal loading, the agarose gel was stained with ethidium bromide.

(Fig. 4B). Transgenic *Arabidopsis* overexpressing the *AtAHL* gene exhibited a high degree of resistance to infection with the pathogen. In the spraying experiment, the percentage of dead leaves and average lesion diameter of spots were smallest in 35S:*AHL* transgenic plants (Fig. 4B).

**Jasmonate induction of *AtAHL* expression.** After treatment with 50  $\mu\text{M}$  MeJA, the *AtAHL* gene transcript level increased within 1 h and reached at the maximum level 24 h after the treatment (Fig. 5). Expressions of cysteine synthase (At3g61440) and ATP-sulfurylase (At4g14680) genes were also induced by MeJA treatment. Expression level of JR2 (*Jasmonate Responsive 2*), an *Arabidopsis* jasmonate-inducible marker gene (Rojo et al., 1999) reached the maximum about 3 h after MeJA treatment.

## Discussion

Increasing the formation of sulfur-containing defense compounds should enhance defense responses in plants. Thus, attempts have been made to engineer sulfate metabolism by generating transgenic plants. Overexpression of endogenous thionins was shown to enhance resistance of *Arabidopsis* against *Fusarium oxysporum* (Epple et al., 1997). Transgenic *Arabidopsis* overproducing aliphatic isopropyl and methylpropyl glucosinolates showed enhanced resistance to *P. carotovorum*, whereas accumulation of *p*-hydroxybenzyl or benzyl glucosinolates produced enhanced resistance toward the bacterial pathogen *Pseudomonas syringae* (Brader et al., 2006). Increased accumulation of aromatic glucosinolates suppressed jasmonate-dependent defenses, as

revealed by enhanced susceptibility of the transgenic plants to the fungus *Alternaria brassicicola* (Brader et al., 2006). Thus, transgenic *Arabidopsis* enriched for a specific glucosinolate altered disease resistance to a specific pathogen.

Reinforcement of the sulfate activation pathway could increase the rate of sulfur flux in the sulfate assimilation pathway, maintaining a wide scope of defense specificity of transgenic plants. PAPS generated in this pathway is a source of the sulfonate group in sulfonation reactions catalyzed by sulfotransferases (Klaassen and Boles, 1997). This molecule is converted to sulfide and eventually incorporated into cysteine. In addition, PAPS is a universal sulfate donor in the conjugation reaction to the activated aldoximes in glucosinolate formation (Sønderby et al., 2010). PAP is a by-product of the PAPS-utilizing reactions, which is dephosphorylated by PAP-nucleotidase to yield 5'-AMP. However, note that accumulation of PAPS and PAP would be toxic to cells. In particular, PAP inhibits sulfotransferases and competes with PAPS for transport into the Golgi apparatus (Klaassen and Boles, 1997). In addition, the methionine auxotrophy of yeast *hal2* mutants was not relieved by inorganic sulfur sources such as sulfite or sulfide (Peng and Verma, 1995). PAP accumulation in yeast *hal2* mutants or in Li<sup>+</sup>-treated wild-type yeast strains inhibits exoribonucleases involved in RNA processing (Dichtl et al., 1997). In a study on plant metabolic reactions, import of the acyl carrier protein precursor into spinach chloroplasts was found to be sensitive to PAP accumulation (Yang et al., 1994).

We postulated that by increasing the PAP degradation reaction in transgenic plants, the rate of sulfur flux in the sulfate assimilation pathway would be increased. Among the known PAP-nucleotidases for gene transformation, AtAHL was the best choice, because this enzyme is highly specific for PAP (Cheong and Kwon, 1999; Gil-Mascarell et al., 1999), whereas yeast HAL2 (Murguía et al., 1996) and *Arabidopsis* SAL1 (Quintero et al., 1996) contain high degree of 3'(2'),5'-diphosphonucleoside 3'(2')-phosphohydrolase activity, which catalyzes the conversion of PAPS back to APS.

Transgenic *AtAHL*-overexpressing *Arabidopsis* plants (Figs. 1 and 2) exhibited enhanced resistance to *P. carotovorum*, whereas *AtAHL* knockout mutant showed increased susceptibility (Fig. 4). *P. carotovorum* causes soft-rot disease by secreting a series of cell wall-degrading enzymes; thus, it is a nonspecific pathogen that is able to infect a wide range of host plants, causing heavy losses in economically important crops (Perombelon and Kelman, 1980). The patterns of expression of several genes involved in defense responses suggested that both jasmonates and salicylic acid play a role in the response of *Arabidopsis* to *P. carotovorum* (Aguilar et al., 2002). It was reported that elicitors isolated from culture filtrates of *P. carotovorum* triggered indole glucosinolates through coordinate induction of the tryptophan biosynthesis genes in *Arabidopsis*, and this response was mediated by jasmonic acid as shown by lack of indole glucosinolates induction in the jasmonate-insensitive mutant *coi1-1* (Brader et al., 2001).

In general, plant defense responses and sulfur metabolism are linked through jasmonic acid signaling (Kruse et al., 2007).

Notably, microarray data indicate that the expression of genes involved in primary and secondary sulfur-related pathways are induced in response to jasmonate treatment in *Arabidopsis* (Jost et al., 2005; Sasaki-Sekimoto et al., 2005; Jung et al., 2007). Jasmonates induce the genes encoding cysteine-rich proteins such as defensins and thionins, which exert an important role in activating a jasmonate-induced defense mechanism against bacterial and fungal pathogens (Broekaert et al., 1995; Hell, 1997; Thomma et al., 2002). Mutation of *SAL1* gene led to deregulated jasmonic acid accumulation in *Arabidopsis* leaves (Rodríguez et al., 2010). Thus, in addition to the observation reported by Brader et al. (2001), it appears that *P. carotovorum* triggers both tryptophan and sulfur metabolisms, increasing the biosynthesis of indole glucosinolates in *Arabidopsis*.

As identified in Northern blot analysis in the present study, *AtAHL* is also a jasmonate-inducible gene (Fig. 5). Therefore, a higher level of *AtAHL* expression in these transgenic plants contributed to the jasmonate-mediated resistance to pathogen infection by accelerating the sulfur flux and at least in part by degrading PAP, a toxic by-product generated from the reductive sulfate assimilation pathway.

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## References

- Aguilar I, Alamillo JM, García-Olmedo F, and Rodríguez-Palenzuela P (2002) Natural variability in the *Arabidopsis* response to infection with *Erwinia carotovora* subsp. *carotovora*. *Planta* **215**, 205–9.
- Brader G, Mikkelsen MD, Halkier BA, and Palva ET (2006) Altering glucosinolate profiles modulates disease resistance in plants. *Plant J* **46**, 758–67.
- Brader G, Tas E, and Palva ET (2001) Jasmonate-dependent induction of indole glucosinolates in *Arabidopsis* by culture filtrates of the nonspecific pathogen *Erwinia carotovora*. *Plant Physiol* **126**, 849–60.
- Broekaert WF, Terras FRG, Cammue BPA, and Osborn RW (1995) Plant defensins: Novel antimicrobial peptides as components of the host defense system. *Plant Physiol* **108**, 1353–8.
- Carpenter CD and Simon AE (1998) Preparation of RNA. *Methods Mol Biol* **82**, 85–9.
- Cheong J-J and Kwon H-B (1999) *Arabidopsis AtAHL* gene encodes a 3'(2'),5'-bisphosphate nucleotidase sensitive to toxic heavy metal ions. *Agric Chem Biotechnol* **42**, 169–74.
- Clough SJ and Bent AF (1998) Floral dip; a simplified method for *Agrobacterium*-mediated transformation of *Arabidopsis thaliana*. *Plant J* **16**, 735–43.
- Cooper RM and Williams JS (2004) Elemental sulfur as an induced antifungal substance in plant defence. *J Exp Bot* **55**, 1947–53.
- Dichtl B, Stevens A, and Tollervey D (1997) Lithium toxicity in yeast is due to the inhibition of RNA processing enzymes. *EMBO J* **16**, 7184–95.
- Droux M (2004) Sulfur assimilation and the role of sulfur in plant metabolism: a survey. *Photosynth Res* **79**, 331–48.

- Epple P, Apel K, and Bohlmann H (1997) Overexpression of an endogenous thionin enhances resistance of *Arabidopsis* against *Fusarium oxysporum*. *Plant Cell* **9**, 509–20.
- Falk KL, Tokuhisa JG, and Gershenzon (2007) The effect of sulfur nutrition on plant glucosinolate content: physiology and molecular mechanisms. *Plant Biol* **9**, 573–81.
- Gil-Mascarell R, López-Coronado JM, Bellés JM, Serrano R, and Rodríguez PL (1999) The *Arabidopsis* HAL2-like gene family includes a novel sodium-sensitive phosphatase. *Plant J* **17**, 373–83.
- Halkier BA and Gershenzon J (2006) Biology and biochemistry of glucosinolates. *Annu Rev Plant Biol* **57**, 303–33.
- Hell R (1997) Molecular physiology of plant sulfur metabolism. *Planta* **202**, 138–48.
- Jost R, Altschmied L, Bloem E, Bogs J, Gershenzon J, Hähnel U et al. (2005) Expression profiling of metabolic genes in response to methyl jasmonate reveals regulation of genes of primary and secondary sulfur-related pathways in *Arabidopsis thaliana*. *Photosynth Res* **86**, 491–508.
- Jung C, Lyou SH, Yeu SY, Kim MA, Rhee S, Kim M et al. (2007) Microarray-based screening of jasmonate-responsive genes in *Arabidopsis thaliana*. *Plant Cell Report* **26**, 1053–63.
- Klaassen CD and Boles JW (1997) The importance of 3'-phosphoadenosine 5'-phosphosulfate (PAPS) in the regulation of sulfation. *FASEB J* **11**, 404–18.
- Kopriva S (2006) Regulation of sulfate assimilation in *Arabidopsis* and beyond. *Ann Bot* **97**, 479–95.
- Kopriva S and Koprivova A (2004) Plant adenosine 5'-phosphosulphate reductase: the past, the present, and the future. *J Exp Bot* **55**, 1775–83.
- Kopriva S, Fritzsche K, Wiedemann G, and Reski R (2007) The Putative moss 3-phosphoadenosine-5-phosphosulfate reductase is a novel form of adenosine-5-phosphosulfate reductase without an iron-sulfur cluster. *J Biol Chem* **282**, 22930–8.
- Koprivova A, Meyers AJ, Schween G, Herschbach C, Reski R, and Kopriva S (2002) Functional knockout of the adenosine 5'-phosphosulfate reductase gene in *Physcomitrella patens* revives an old route of sulfate assimilation. *J Biol Chem* **277**, 32195–201.
- Kruse C, Jost R, Lipschis M, Kopp B, Hartmann, and Hell R (2007) Sulfur-enhanced defence: effects of sulfur metabolism, nitrogen supply, and pathogen lifestyle. *Plant Biol* **9**, 608–19.
- Leyh TS (1993) The physical biochemistry and molecular genetics of sulfate activation. *Crit Rev Biochem Mol Biol* **28**, 515–42.
- Murguía JR, Bellés JM, and Serrano R (1996) The yeast HAL2 nucleotidase is an *in vivo* target of salt toxicity. *J Biol Chem* **271**, 29029–33.
- Peng Z and Verma DP (1995) A rice HAL2-like gene encodes a Ca<sup>2+</sup>-sensitive 3'(2')-phosphohydrolase and complements yeast *met22* and *Escherichia coli cysQ* mutations. *J Biol Chem* **270**, 29105–10.
- Perombelon MCM and Kelman A (1980) Ecology of the soft rot Erwinias. *Annu Rev Phytopathol* **18**, 361–87.
- Quintero FJ, Garcíadeblas B, and Rodríguez-Navarro A (1996) The *SAL1* gene of *Arabidopsis*, encoding an enzyme with 3'(2'),5'-bisphosphate nucleotidase and inositol polyphosphate 1-phosphatase activities, increases salt tolerance in yeast. *Plant Cell* **8**, 529–37.
- Rausch T and Wachter A (2005) Sulfur metabolism: a versatile platform for launching defence operations. *Trends Plant Sci* **10**, 503–9.
- Rodríguez VM, Chetelat A, Majcherczyk P, and Farmer EE (2010) Chloroplastic phosphoadenosine phosphosulfate metabolism regulates basal levels of the prohormone jasmonic acid in *Arabidopsis* leaves. *Plant Physiol* **152**, 1335–45.
- Rojo E, León J, and Sánchez-Serrano JJ (1999) Cross-talk between wound signaling pathways determines local versus systemic gene expression in *Arabidopsis thaliana*. *Plant J* **20**, 135–42.
- Sasaki-sekimoto Y, Taki N, Obayashi T, Aono M, Matsumoto F, Sakurai N et al. (2005) Coordinated activation of metabolic pathways for antioxidants and defence compounds by jasmonates and their roles in stress tolerance in *Arabidopsis*. *Plant J* **44**, 653–68.
- Sønderby IE, Geu-Flores F, and Halkier BA (2010) Biosynthesis of glucosinolates - gene discovery and beyond. *Trends Plant Sci* **15**, 283–90.
- Takahashi H, Kopriva S, Giordano M, Saito K, and Hell R (2011) Sulfur Assimilation in Photosynthetic Organisms: Molecular Functions and Regulations of Transporters and Assimilatory Enzymes. *Annu Rev Plant Biol* **62**, 157–84.
- Thomma BPHJ, Cammue BPA, and Thevissen K (2002) Plant defensins. *Planta* **216**, 193–202.
- Yang L-M, Fernandez MD, and Lamppa GK (1994) Acyl carrier protein (ACP) import into chloroplasts: Covalent modification by a stromal holo ACP synthase is stimulated by exogenously added CoA and inhibited by adenosine 3',5'-bisphosphate. *Eur J Biochem* **224**, 743–50.