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Crosstalk between biotic and abiotic stress responses in tomato is mediated by the *AIM1* transcription factor

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Summary

Plants deploy diverse molecular and cellular mechanisms to survive in stressful environments. The tomato (*Solanum lycopersicum*) *abscisic acid-induced myb1* (*SlAIM1*) gene encoding an R2R3MYB transcription factor is induced by pathogens, plant hormones, salinity and oxidative stress, suggesting a function in pathogen and abiotic stress responses. Tomato *SlAIM1* RNA interference (RNAi) plants with reduced *SlAIM1* gene expression show an increased susceptibility to the necrotrophic fungus *Botrytis cinerea*, and increased sensitivity to salt and oxidative stress. Ectopic expression of *SlAIM1* is sufficient for tolerance to high salinity and oxidative stress. These responses correlate with reduced sensitivity to abscisic acid (ABA) in the *SlAIM1* RNAi, but increased sensitivity in the overexpression plants, suggesting *SlAIM1*-mediated ABA responses are required to integrate tomato responses to biotic and abiotic stresses. Interestingly, when exposed to high root-zone salinity levels, *SlAIM1* RNAi plants accumulate more Na^+, whereas the overexpression lines accumulate less Na^+ relative to wild-type plants, suggesting that SlAIM1 regulates ion fluxes. Transmembrane ion flux is a hallmark of early responses to abiotic stress and pathogen infection preceding hypersensitive cell death and necrosis. Misregulation of ion fluxes can result in impaired plant tolerance to necrotrophic infection or abiotic stress. Our data reveal a previously uncharacterized connection between ABA, Na^+ homeostasis, oxidative stress and pathogen response, and shed light on the genetic control of crosstalk between plant responses to pathogens and abiotic stress. Together, our data suggest *SlAIM1* integrates plant responses to pathogens and abiotic stresses by modulating responses to ABA.

Keywords: tomato, *Solanum lycopersicum*, ABA-induced R2R3MYB, *Botrytis cinerea*, salt stress.

Introduction

Plant responses to biotic and abiotic stresses involve a network of molecular mechanisms that vary depending on the nature of the pathogen or the stress signal. Plant responses to necrotrophic fungi are complex, involving diverse genetic and molecular mechanisms, and vary depending on the primary mechanism of the pathogen virulence (Wolpert *et al.*, 2002; Glazebrook, 2005). Broad host necrotrophic fungi produce toxins, cell-wall degrading enzymes (CWDEs) and reactive oxygen intermediates (ROIs) that determine the severity of disease (Edlich *et al.*, 1989; Tiedemann, 1997; Muckenschnabel *et al.*, 2002). These disease factors cause electrolyte leakage, changes in ion fluxes, cell death and other stress responses, underlining the similarities in plant responses to microbial necrotrophy and abiotic stresses. Exposure to abiotic stress, in some cases, enhances resistance to pathogens indicative of crosstalk between biotic and abiotic stress signaling (Bowler and Fluhr, 2000). Induced resistance common to both biotic and abiotic stresses has also been documented (Zimmerli *et al.*, 2000; Jakab *et al.*, 2005; Ton *et al.*, 2005).

Distinct response pathways that regulate plant responses to diverse environmental signals have been extensively described. However, recent studies suggest a greater coordination of plant responses to pathogens and abiotic stresses, including the expression of overlapping sets of genes in response to infection and abiotic stresses (Cheong *et al.*, 2002; AbuQamar *et al.*, 2006; Fujita *et al.*, 2006). The plant hormones ethylene (ET), salicylate (SA), jasmonate

(JA) and abscisic acid (ABA) act synergistically or antagonistically to regulate plant responses to pathogens and abiotic stress factors. In addition, ROIs and secondary messengers, such as calcium, modulate plant responses to diverse environmental signals (Bowler and Fluhr, 2000). Regulatory proteins, including transcription factors, protein kinases and diverse post-translational mechanisms, regulate responses to plant hormones and ROIs, both of which are central to plant responses to biotic and abiotic stresses (Liu and Zhang, 2004; Rentel *et al.*, 2004). The accumulation of ROIs precedes cell death and is associated with resistance to biotrophic pathogens (Lamb and Dixon, 1997). Cell death promotes plant susceptibility to some necrotrophic fungi (Govrin and Levine, 2000). ROIs also mediate abiotic stress-induced cell death (Torres *et al.*, 2002), and Arabidopsis NADPH oxidases, primary sources of ROIs, control responses to the plant stress hormone ABA (Kwak *et al.*, 2003). Thus, plant responses to environmental signals are regulated by a network of intracellular pathways.

Abscisic acid regulates the plant response to drought, low temperature and osmotic stress. Recently, ABA has emerged as a positive or negative regulator of disease resistance, depending on the nature of the host–pathogen interaction (Anderson *et al.*, 2004; Lorenzo *et al.*, 2004; Mauch-Mani and Mauch, 2005). ABA deficiency in tomato and impaired ABA responses in Arabidopsis result in increased resistance to *Botrytis cinerea*, and other necrotrophic pathogens, as a result of the reduced ABA signaling but increased JA- or ET-responsive gene expression (Audenaert *et al.*, 2002). The *enhanced response to ABA3* (*ERA3*) gene is allelic to *EIN2* (Ghassemian *et al.*, 2000), which is required for resistance to some necrotrophic fungi (Thomma *et al.*, 1999). In addition, resistance to the necrotrophic oomycete *Pythium irregulare* and to the bacterial necrotroph *Ralstonia solanacearum* requires ABA synthesis and responses indicating a positive role for ABA in disease resistance (Adie *et al.*, 2007b; Hernandez-Blanco *et al.*, 2007). ABA also controls stomatal closure during pathogen invasion, thereby regulating microbial access to plant tissues (Melotto *et al.*, 2006). ABA regulation of stomatal closure is dependent on ROIs (Kwak *et al.*, 2003). Furthermore, abiotic and biotic stress responses often converge into the mitogen-activated protein kinase (MAPK) signaling in Arabidopsis. Arabidopsis MPK3 and MPK4 function in abiotic stress and basal defense responses (Nuhse *et al.*, 2000; Asai *et al.*, 2002; Jonak *et al.*, 2002; Rentel *et al.*, 2004; Veronese *et al.*, 2006). Arabidopsis OXI1 regulates the activation of MPK3 and MPK6 by ROIs, and is also required for pathogen resistance (Rentel *et al.*, 2004). Thus, pathogen and stress response signaling share significant regulatory mechanisms, with complex interactions between responses to plant hormones, pathogens, abiotic stresses and ROIs.

The genetic factors and molecular mechanisms that mediate biotic and abiotic stress responses of tomato (*Solanum lycopersicum*) are not known. Here, the role of tomato *abscisic acid-induced MYB1* (*AIM1*) transcription factor (*SlAIM1*) in pathogen and abiotic stress responses is described. Tomato *AIM1* RNA interference (RNAi) plants, with a reduced expression of *SlAIM1*, show an increased susceptibility to *B. cinerea*, a sensitivity to salt stress, but a reduced sensitivity to ABA. Overexpression of *SlAIM1* enhanced ABA sensitivity, and the tolerance to salt and oxidative stress, but did not improve resistance to *B. cinerea.* These phenotypes suggest that *B. cinerea* resistance is a complex trait in tomato, consistent with the multiplicity of the *B. cinerea* disease factors. Interestingly, elemental profiling of leaf tissues reveals that *SlAIM1* RNAi plants exposed to high root-zone salinity levels accumulate more Na^+, whereas the overexpression line has reduced concentrations. Thus, *SlAIM1* may control an Na^+ removal or exclusion mechanism. Our data suggest that *SlAIM1* mediates responses to biotic and abiotic stresses, and links plant responses to plant hormones, ROIs, microbial infection and abiotic stress factors.

Results

Identification and characterization of the tomato SlAIM1 *gene*

The tomato *SlAIM1* transcription factor was cloned based on sequence homology to the MYB DNA binding domains of Arabidopsis *Botrytis-susceptible 1* (*BOS1*). *BOS1* encodes an R2R3MYB transcription factor required for resistance to necrotrophic pathogens, and for tolerance to some abiotic stresses (Mengiste *et al.*, 2003). *SlAIM1*-specific gene expression is undetectable in leaf tissues grown under normal conditions, but is induced by *B. cinerea* in tomato leaves that are indicative of a disease resistance function (Figure 1a). Expression was detectable by 24 h after inoculation, and increased further after 48–72 h. *SlAIM1* was also induced by the bacterial pathogen *Pseudomonas syringae*, with a significant increase starting at 12 h after inoculation (Figure 1b). SA and the natural precursor of ethylene synthesis, 1-aminocyclopropane-1-carboxylic acid (ACC), failed to induce *SlAIM1*. ABA and NaCl induced *SlAIM1* significantly, whereas paraquat, a herbicide that causes oxidative stress, and MeJA only slightly induced *SlAIM1* (Figure 1c). Infiltration with buffer alone did not induce the *SlAIM1* transcript and, thus, the induction is specific to pathogens, a subset of plant hormones and stress factors.

SlAIM1 is predicted to be a member of a large gene family in tomato, based on the available knowledge from other genomes. A DNA blot of tomato genomic DNA digested with different restriction enzymes and hybridized to a gene-specific region of *SlAIM1*, shows a single hybridizing band consistent with a single-copy gene in the tomato genome (Figure 1d). In addition, the full-length *SlAIM1* was transla-

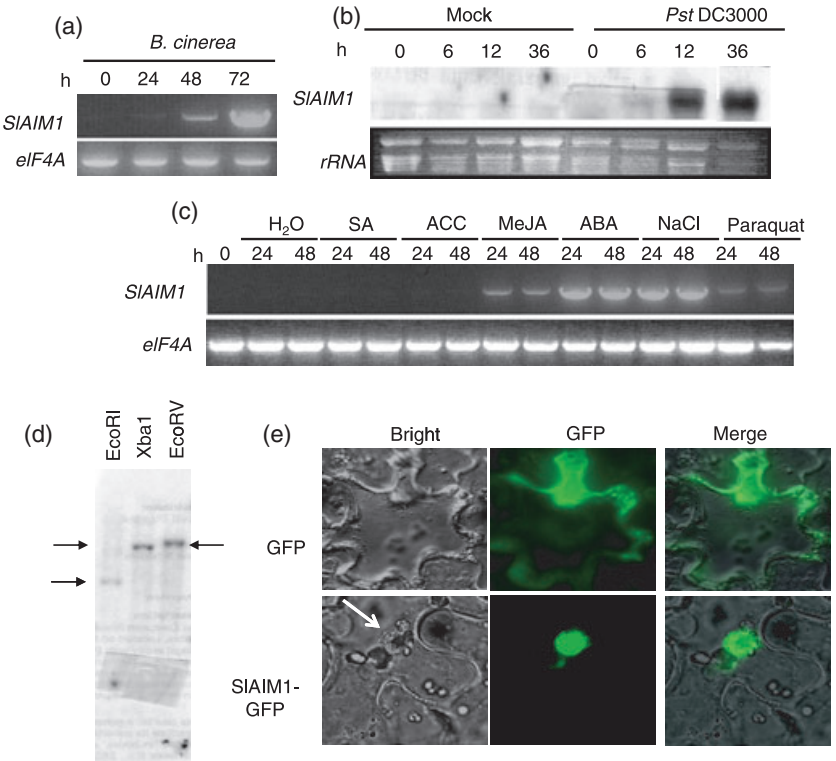

Figure 1. *SlAIM1* gene expression, copy number and subcellular localization.
Expression of *SlAIM1* in response to: (a) *Botrytis cinerea*; (b) *Pseudomonas syringae* pv. *tomato* DC3000 (*Pst*DC3000); and (c) plant hormones, salinity and oxidative stress (paraquat).
(d) DNA blot showing a single copy of the *SlAIM1* gene in the tomato genome.
(e) Subcellular localization of the SlAIM1-GFP fusion protein in *Nicotiana benthamiana* leaves. The arrow in the bright-field image shows the position of the nucleus.
In panels (a–c), tomato CastlemartII wild-type plants were drop-inoculated with *B. cinerea*, infiltrated with *Pst*DC3000 or sprayed with paraquat and plant hormones. In (a) and (c), RT-PCR was performed as described in the Experimental procedures. The constitutively expressed tomato translation initiation factor gene (*eIF4A*) was used as a control. NaCl induction was performed by watering plants with a 200 mM NaCl solution. In (b), the total RNA (15 μg) was loaded per lane. In (d), the DNA blot was hybridized to the gene-specific region of *SlAIM1*. The experiments were repeated at least three times with similar results. h, hours post-inoculation.

tionally fused with green fluorescent protein (GFP), and the chimeric protein was transiently expressed in *Nicotiana benthamiana* leaf tissues through *Agrobacterium* infiltration, to determine the subcellular localization of SlAIM1. The SlAIM1-GFP fusion protein only localized to the nucleus when it was expressed in *N. benthamiana* epidermal cells, consistent with its predicted DNA-binding functions, whereas cells expressing GFP alone (control, top row) exhibited a diffuse signal in both the cytosol and the membrane (Figure 1e). Thus, the data suggest that SlAIM1 is a nuclear protein and that the gene is regulated by plant hormones, pathogens and oxidative stress, indicative of a function in stress response signaling.

Sequence and phylogenetic analyses of tomato AIM1 and its relationship with other R2R3 MYB transcription factors

The *SlAIM1* cDNA contains a 732-bp-long open reading frame (ORF) encoding a protein of 244 amino acids. The predicted SlAIM1 protein contains the R2R3MYB DNA binding domains close to the N-terminal sequence (positions 19–71 aa and 72–121 aa), and a C-terminal region of unknown function (Figure S1). SlAIM1 also contains the SANT domain, a putative DNA binding module recently found in diverse proteins with functions in chromatin remodeling (Zhang *et al.*, 2006). The SANT domain falls within the predicted R2 MYB repeat, and its functional significance in plants is not clear. SlAIM1 shares high sequence identity to Arabidopsis MYB78 (At5g49620), BOS1 (MYB108, At3g06490), MYB112 (At1g48000), MYB2 (At2G47190) and two dehydration-induced MYB-related proteins, CPM5 and CPM10, from the resurrection plant *Craterostigma plantagineum* (Iturriaga *et al.*, 1996) (Figure S1a,b). The R2 and R3 DNA binding domains and the short segment following the R3 domain show high sequence conservation, with 62–84% identity shared between SlAIM1 and the related MYB proteins. SlAIM1 shows the highest sequence relatedness to Arabidopsis MYB78 (84% identity), followed by CPM10 (83%) and BOS1 (82%) in the N-terminal conserved region covering the MYB domains. The SlAIM1 protein carries multiple deletions compared with the closely related R2R3 MYB proteins. *SlAIM1* encodes the smallest protein (29.4 kDa), whereas *AtBOS1* encodes the largest protein (37 kDa), of the closely related MYBs (Figure S1b) (Stracke *et al.*, 2001). The C-terminal sequence of SlAIM1 has no significant sequence identity to BOS1 and the other related MYBs. The Arabidopsis BOS1 autoactivates in yeast two-hybrid assays, with the activation domain of BOS1 mapped to the C-terminal 50 amino acid sequence (H. Luo, F. Song, K. Laluk, T. Mengiste, unpublished data). SlAIM1 shows no autoactivation (data not shown), suggesting that its transcriptional regulatory mechanism is different from BOS1, and that the activation domains of BOS1 and SlAIM1 are divergent.

The Arabidopsis genome contains ~135 R2R3MYB genes, with 22 subfamilies defined based on the conservation of the MYB DNA binding repeats, and a short sequence that is

C-terminal to the R3 domain (Stracke *et al.*, 2001). One subfamily representing a phylogentic clade contains Arabidopsis BOS1, MYB78, MYB112, MYB116, MYB2 and MYB62 (Stracke *et al.*, 2001). Phylogenetic analysis places SlAIM1 in this clade (Figure S1c). Among the genes in this clade, Arabidopsis T-DNA insertion alleles in *MYB78* and *MYB112* show no altered responses to *B. cinerea* and *Alternaria brassicicola*, whereas *bos1* shows increased susceptibility (Mengiste *et al.*, 2003) (Figure S2). The disease resistance function of MYB112, MYB62 and MYB2 are not determined. MYB2 regulates Arabidopsis responses to salinity, ABA and drought (Abe *et al.*, 2003), and shares 67% identity to SlAIM1 in the R2 and R3 MYB domains. *CPM5* and *CPM10* genes are induced by drought stress and ABA in *C. plantagineum* tissues, and have been associated with plant stress responses (Iturriaga *et al.*, 1996). Arabidopsis *BOS1*, *MYB2* and tomato *AIM1* genes are all induced by an overlapping set of factors, including ABA, NaCl, *B. cinerea* and *P. syringae* (Table S1). Thus, SlAIM1 may be functionally related to Arabidopsis BOS1, MYB2 and *C. plantagineum* CPM10.

Tomato AIM1 *is required for resistance to* B. cinerea

To determine the function of *SlAIM1*, tomato lines with reduced *SlAIM1* gene expression were generated by expressing double-strand RNA (dsRNA) corresponding to the gene-specific region of *SlAIM1. SlAIM1* expression was reduced in several transgenic tomato lines relative to wild-type plants (Figure 2a). Tomato *SlAIM1* RNAi lines 1 and 8 had the greatest reduction in *SlAIM1* gene expression, and were used for the experiments described in this report. The *SlAIM1* RNAi plants do not show any developmental growth defects when grown under normal horticultural conditions. However, 2 days after challenge with *B. cinerea*, *SlAIM1* RNAi plants showed an increased susceptibility, with larger disease lesions than the CastlemartII wild-type plants (Figure 2b,c). This was accompanied by a slight increase in fungal growth, as measured by the levels of the *B. cinerea ActinA* gene transcript (Benito *et al.*, 1998) (Figure 2d). In addition, we suppressed the *SlAIM1* gene expression through virus-induced gene silencing (VIGS) (Liu *et al.*, 2002) in the cherry tomato cultivar Micro-Tom. In whole-

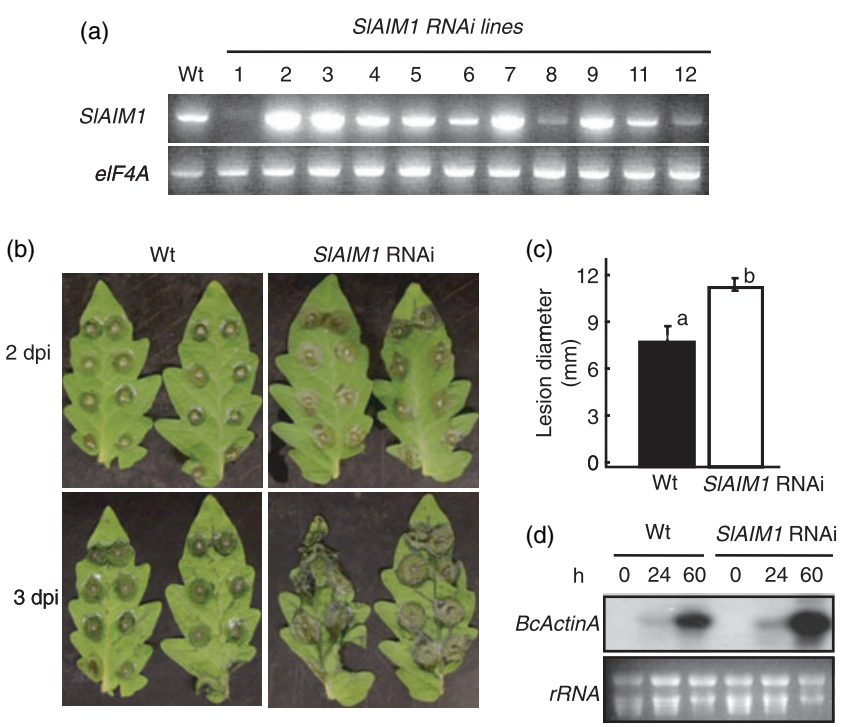

Figure 2. *SlAIM1* is required for the full resistance of tomato plants to *Botrytis cinerea*.
(a) RT-PCR showing *SlAIM1* transcript levels in *SlAIM1* RNAi lines.
(b) *SlAIM1* RNAi plants show increased susceptibility to *B. cinerea*.
(c) Disease lesion size in *B. cinerea*-inoculated leaves at 2 days after inoculation.
(d) RNA gel blot showing accumulation of *B. cinerea ActinA* mRNA as a measure of fungal growth in inoculated tomato leaves.
In (a), RNA samples were extracted from leaves at 3 days after inoculation with *B. cinerea*. In (d), the total RNA (15 μg) was loaded per lane. In (c), the data represent the mean ± SE from a minimum of 60 lesions. Both an analysis of variance (ANOVA) and Duncan's multiple range test were performed to determine the statistical significance of the mean disease lesion sizes using SAS software (SAS, 1999). Bars with different letters are significantly different from each other ($P = 0.05$). Experiments were repeated at least three times with similar results. The disease symptoms in (b) are representative of *SlAIM1* RNAi lines 1 and 8. The data from the *SlAIM1* RNAi line 1 is presented in panels (c) and (d). Ethidium bromide staining of rRNA or the amplification of the tomato *elongation factor 4A* (*elF4A*) were used as controls. Abbreviations: *BcActinA, Botrytis cinerea ActinA* gene; h, hours post-inoculation.

plant disease assays *SlAIM1* VIGS plants showed severe disease symptoms, with extensive tissue damage at 3 days post-inoculation (dpi) with *B. cinerea*, compared with the wild-type cultivar, thereby confirming the data from the RNAi plants (Figure S3).

SlAIM1 RNAi plants were tested for responses to the virulent strain of the bacterial pathogen *P. syringae* pv. tomato. Plants were inoculated by infiltration, spray inoculation or seedling incubation with bacterial suspension, as described by Zipfel *et al.* (2004) and Uppalapati *et al.* (2008). There was no difference observed in both symptom development and bacterial growth between *SlAIM1* RNAi and the wild-type plants, regardless of the inoculation method (data not shown). Thus, the disease resistance function of *SlAIM1* does not extend to *P. syringae*.

Tomato lines that overexpress *SlAIM1* (*35S:SlAIM1*) were generated in the Micro-Tom cultivar, and two transgenic lines (35S:*SlAIM1* lines 4 and 7) that have high *SlAIM1* gene expression were selected (Figure S4a). The 35S:*SlAIM1* tomato plants were comparable with the wild-type plants in the level of *B. cinerea* resistance (Figure S4b).

Expression of tomato defense genes during B. cinerea *infection and wounding*

The expression of tomato defense genes was studied to determine whether the *B. cinerea* susceptibility of *SlAIM1* RNAi plants is related to tomato defense pathways affecting *B. cinerea* resistance (Schilmiller and Howe, 2005; AbuQamar *et al.*, 2008). The uninfected tomato plants express low levels of tomato *protease inhibitor-II* (*PI-II)* and *Allen oxide synthase* 2 (*AOS2*) genes, normally associated with wound and JA responses. The *PI-II* and *AOS2* genes were strongly induced in response to *B. cinerea* infection and mechanical wounding, independent of *SlAIM1* (Figure 3a). Thus, the enhanced susceptibility of *SlAIM1* RNAi plants to *B. cinerea* is independent of at least part of the JA/wound response pathways leading to the expression of these genes. The *ACC synthase* (*ACS*) gene expression is induced to the same level in both the wild-type and *SlAIM1* RNAi plants in response to *B. cinerea* and wounding, suggesting that *SlAIM1* acts independently of the ET-dependent defense response pathway in tomato. The *SlAIM1* gene is also not required for the *B. cinerea* and wound induced expression of the *pathogenesis related protein 1* (*PR1)* gene, a molecular marker for SA-mediated defense responses.

The tomato JA and wound response mutants *suppressor of prosystemin-mediated responses 1* and *2* (*spr1* and *spr2*), *jasmonate-insensitive 1* (*jai1*), *defenseless 1* (*def1*) and *acyl-CoA oxidase* (*acx1*) show impaired JA and wound responses, and/or increased susceptibility to *B. cinerea* (Schilmiller and Howe, 2005; AbuQamar *et al.*, 2008). *SlAIM1* was expressed at wild-type levels in these mutants in response to *B. cinerea* infection (Figure 3b). Thus, the data suggest that the expression of *SlAIM1* during *B. cinerea* infection and its *B. cinerea* resistance function is likely to be independent of JA and/or wound response pathways in tomato.

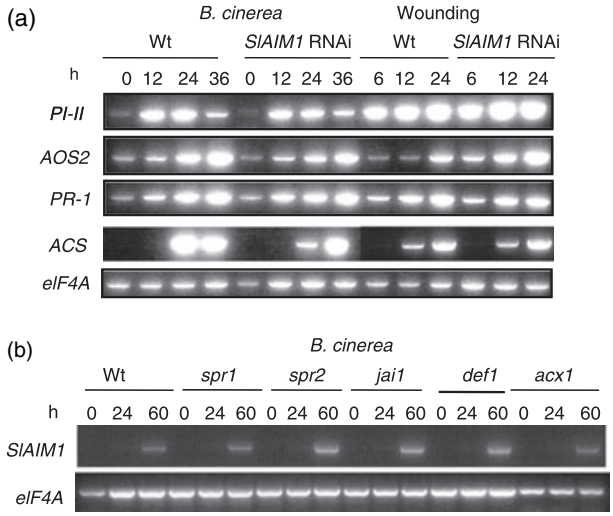

Figure 3. Expression of jasmonate (JA)/wound response genes in Castlemart-tll wild-type (Wt) and *SlAIM1* RNAi plants.
RT-PCR showing expression of tomato (a) *PI-II*, *AOS2*, *ACS* and *PR-1* genes during *Botrytis cinerea* infection and wounding.
(b) *SlAIM1* gene expression in tomato JA/wound response mutants in response to *B. cinerea.*
Experiments were repeated at least three times with similar results. Abbreviations: *ACS*, *ACC synthase*; *AOS2*, *Allen oxide synthase 2*; h, hours after inoculation with *B. cinerea* or wounding treatment; *PI-II*, *protease inhibitor-II*; *PR1*, *pathogenesis-related protein 1*.

SlAIM1 *is required for responses to ABA but not to other plant hormones*

Hormone sensitivity of seedlings was assayed to determine if impaired defense in the *SlAIM1* RNAi plants results from impaired hormone-related functions mediating plant defense and abiotic stress responses. *SlAIM1* RNAi plants show no altered sensitivity to ET and MeJA (data not shown). In contrast, the *SlAIM1* RNAi seedlings show reduced sensitivity of root elongation to ABA (Figure 4a,b). The *SlAIM1* RNAi seeds also showed reduced germination and growth when directly plated on media containing ABA (Figure 4c; data not shown). By contrast, the 35S:*SlAIM1* plants showed increased sensitivity to ABA (Figure 4d). ABA mediates plant responses to biotic and abiotic stresses, and these data suggest that *SlAIM1* is required for ABA responses and *B. cinerea* resistance.

SlAIM1 *is sufficient for increased tolerance to salinity and oxidative stress*

The *SlAIM1* RNAi plants were tested for sensitivity of seed germination and seedling growth to increased salinity

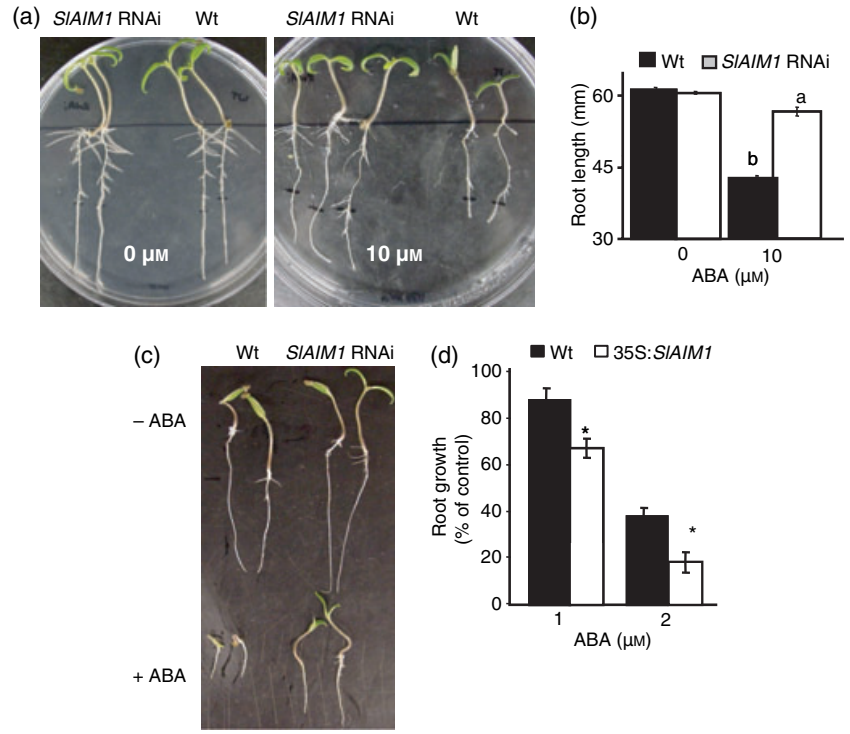

Figure 4. Tomato *AIM1* is required for abscisic acid (ABA) responses.
(a) Root growth sensitivity and (b) root length of Castlemartll wild-type (Wt) and *SlAIM1* RNAi seedlings in the presence or absence of ABA.
(c) Germination of Castlemartll wild-type (Wt) and *SlAIM1* RNAi seedlings in the presence or absence of ABA.
(d) Root growth of Micro-Tom wild-type and 35S:*SlAIM1* seedlings relative to controls.
In panel (a), seeds were germinated and grown for 4 days on plain MS plates and transferred to MS plates with or without ABA. In panel (b), seedlings with ~2–cm-long roots were incubated vertically on MS medium supplemented with 10 μM ABA and their growth was measured. Bars with different letters are significantly different from each other at the level $P = 0.05$. Error bars represent the standard deviations ($n = 9$). The pictures in (a) and measurements in (b) were taken 2 days after transfer to ABA. In panel (c), surface-sterilized seeds were directly plated on MS medium containing no ABA (–ABA) or 2 μM ABA (+ABA). Pictures were taken 6 days after plating seeds. In panel (d), the data represent root growth as a percentage of root growth on plain MS media. Statistical analysis was performed as described in the legend for Figure 2. In (d), the asterisk indicates that the data on root growth show statistically significant differences from the wild-type plants. The experiments were repeated at least three times with similar results. The data in (a)–(c) are from representative samples of *SlAIM1* RNAi lines 1 and 8, and the data in (d) are from the transgenic line 35S:*SlAIM1* line 7.

(NaCl). Compared with the wild type, the *SlAIM1* RNAi plants have significantly reduced seed germination and growth on medium containing high salt concentrations (Figure S5a). At 50 mM NaCl, both the wild-type and *SlAIM1* RNAi plants fully germinated, but the *SlAIM1* RNAi plants had a drastically reduced shoot and radical growth after germination. At 100 mM, the germination of *SlAIM1* RNAi seeds was mostly inhibited. When seedlings pre-germinated on MS media were transferred to media containing 125 mM NaCl, the root growth of *SlAIM1* RNAi seedlings was significantly reduced relative to wild-type plants (Figure S5b,c).

The salinity response assay was repeated under glasshouse conditions (see Experimental procedures). *SlAIM1* RNAi seedlings exposed to high salinity show a clear pattern of salt sensitivity, with reduced shoot and root biomass, chlorosis and tissue collapse, in a dose-dependent manner (Figures 5 and S6). At 150 and 250 mM NaCl, stress-induced symptoms were visible as early as 5 days after salt treatment (Figure S6a). At 10 days, the *SlAIM1* RNAi plants watered with 250 mM NaCl were dead. At 14 days, *SlAIM1* plants watered with 200 mM NaCl exhibited reduced growth, and increased necrosis and chlorosis (Figure S6b). At 21 days, at most concentrations tested, the RNAi plants show chlorotic symptoms, total collapse of tissue and reduced shoot biomass (Figure 5a,b). A similar dose-dependent reduction in total root biomass was observed in *SlAIM1* RNAi plants, as compared with the wild-type plants (Figure 5c,d).

The 35S:*SlAIM1* plants generated in the Micro-Tom genetic background were assayed for increased tolerance by extended exposure to salt stress. In the absence of stress, the tomato 35S:*SlAIM1* plants show reduced growth compared with the wild-type cultivar (Figure 6). When exposed to salt, the biomass accumulation of the wild-type plants was reduced in a dose-dependent manner (Figure 6a,b). By contrast, the growth of 35S:*SlAIM1* plants was less affected by increasing salt concentrations. The wild-type plants show significant and subsequent reductions in shoot growth starting at 100 mM NaCl, relative to untreated controls, whereas the 35S:*SlAIM1* plants exhibited no significant decline in shoot biomass over most NaCl concentrations

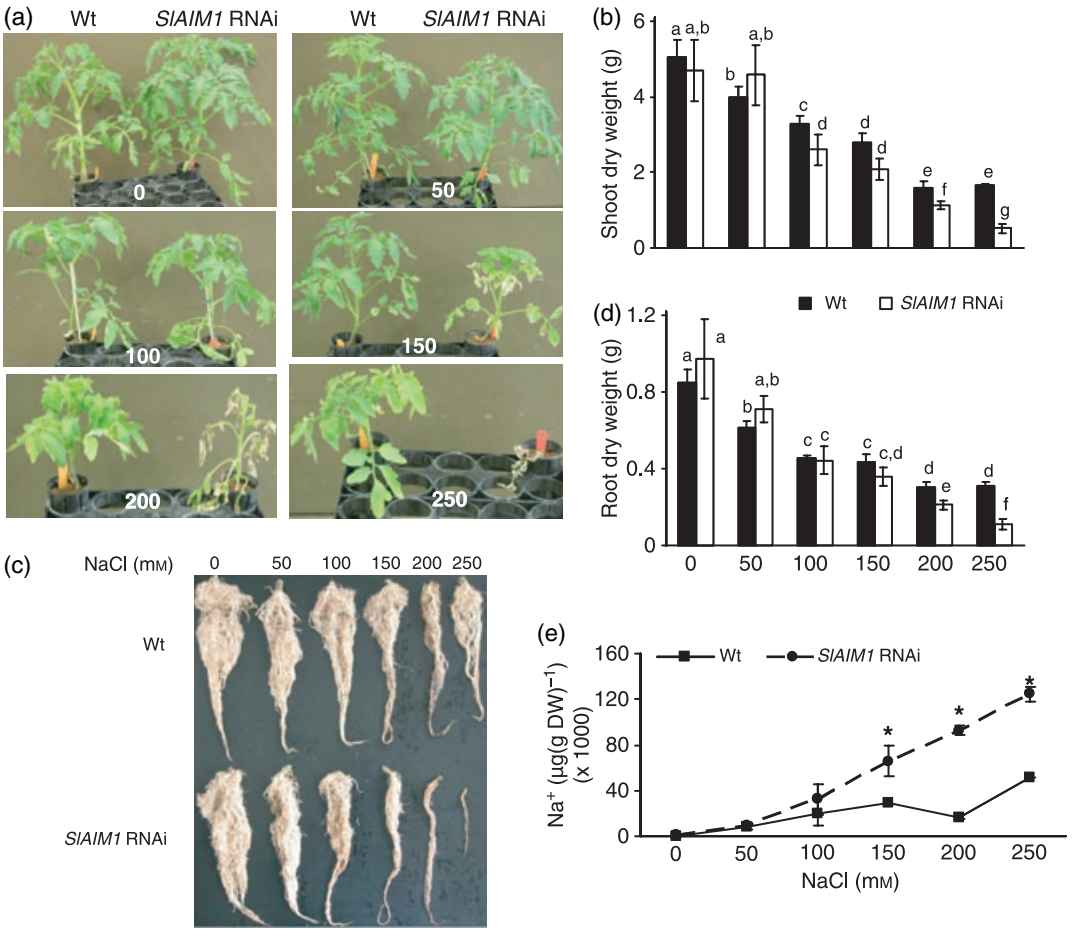

Figure 5. *SlAIM1* is required for tolerance to salt stress.
(a) Salinity-induced stress symptoms in vegetative tissues.
(b) The dry weight of shoot matter (n = 9).
(c) Salinity-induced changes in root biomass.
(d) Weight of root dry matter (n = 9).
(e) Leaf tissue Na^+ concentrations of Castlemartll wild-type and *SlAIM1* RNAi plants (n = 10) exposed to salt.
The pictures in (a) and (c) are from representative samples of *SlAIM1* RNAi lines 1 and 8, and were taken at 21 days after the start of the salt treatment. The measurements from *SlAIM1* RNAi line 1 are presented in (b), (d) and (e). In (b), (d) and (e), an analysis of variance (ANOVA) and a Duncan's multiple range test were performed to determine the statistical significance of differences of the mean dry weights using the SAS software (SAS, I, 1999). The bars with different letters are significantly different from each other (P = 0.05). In (e), the asterisk indicates statistically significant differences in the mean Na^+ concentrations between wild-type and *SlAIM1* RNAi plants (P = 0.05).

tested. When grown at salinity concentrations of 200 and 250 mM, the wild-type Micro-Tom had significantly reduced root and shoot biomass, whereas the 35S:*SlAIM1* was less affected (Figure 6a–c). Interestingly, 35S:*SlAIM1* plants showed an increasing root length under increasing salt stress (Figure S7). In a different assay, plants were continuously grown under different concentrations of salinity, starting at 10 days after germination. The wild-type plants were completely killed between 25–30 days, whereas the overexpression line survived (Figure S8). Thus, *SlAIM1* is sufficient to confer increased resistance to high salinity. The *SlAIM1* RNAi and 35S:*SlAIM1* plants show no altered germination and growth in the presence of mannitol and sorbitol (data not shown). Thus, sensitivity to salinity is likely to result from ionic effects of the salinity stress, rather than osmotic effects.

Elemental profiling of leaf tissue (Lahner *et al.*, 2003) revealed that *SlAIM1* RNAi leaves accumulate significantly higher concentrations of Na^+ than the corresponding wild-type plants (Figure 5e). The 35S:*SlAIM1* plants show significantly reduced Na^+ concentrations, consistent with the increased tolerance of the 35S:*SlAIM1* line to high salinity (Figure 6d). All other elements measured did not vary significantly between the RNAi, 35S:*SlAIM1* and wild-type plants (Figures S9 and S10).

In addition, in media containing 3 mM hydrogen peroxide (H_2O_2), *SlAIM1* RNAi plants show a severe reduction in seedling growth. At 5 mM H_2O_2, *SlAIM1* RNAi plants failed to

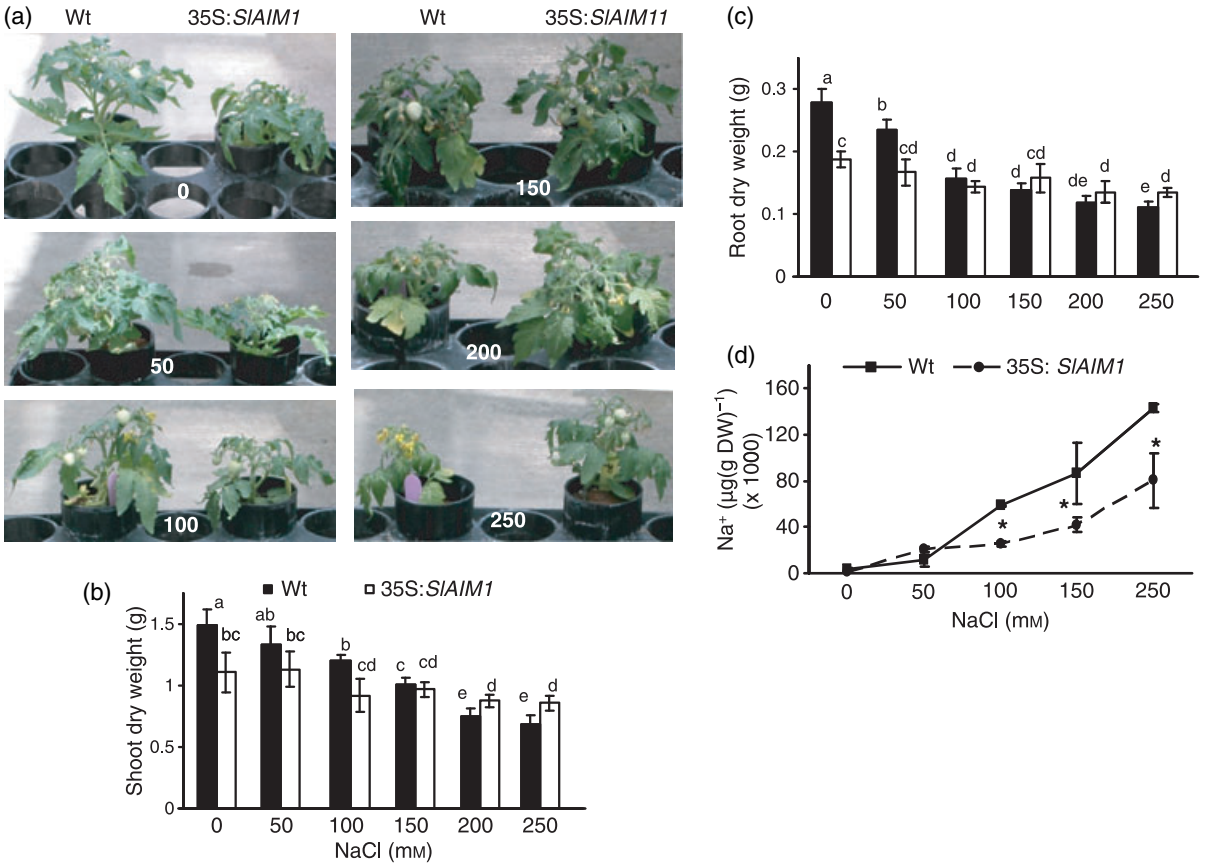

Figure 6. *SlAIM1* is sufficient for increased resistance to salt stress.
(a) Responses of Micro-Tom wild-type and 35S:*SlAIM1* plants to various concentrations of salt.
(b) Shoot (n = 9) and (c) root dry weights (n = 9), and (d) Na^+ concentration in leaf tissue (n = 10), of 35S:*SlAIM1* plants.
The pictures in (a) are from representative samples of 35S:*SlAIM1* lines 4 and 7, at 33 days after the initiation of salinity treatment. The measurements taken from transgenic line 35S:*SlAIM1* line 7 are presented in (b), (c) and (d). Statistical analysis was performed as described in the legend for Figure 5. In (b) and (c), the bars with different letters are significantly different from each other (P = 0.05). In (d), the asterisks indicate that the mean values are significantly different from each other (P = 0.05).

grow normally following germination, producing only a small radical with no shoot emerging, whereas the wild-type plant had fully germinated, with some shoot and limited root growth (Figure 7a). The 35S:*SlAIM1* seedlings were more resistant to H_2O_2 than the wild-type plants (Figure 7b). The data suggest that *SlAIM1* is sufficient to confer tolerance to oxidative stress caused by H_2O_2.

The *SlAIM1* RNAi and 35S:*SlAIM1* plants do not show altered expression of the tomato gene *NHX1*, involved in intracellular K^+ and Na^+ transport (Venema *et al.*, 2003), superoxide dismutase, implicated in tolerance to various environmental stresses (Seong *et al.*, 2007), or the tomato ABA- and salt-inducible dehydrin gene *TAS14* (Godoy *et al.*, 1990). The tomato Na^+/H^+ antiporter *SOS1* gene was also normally expressed in the *SlAIM1* RNAi and 35S:*SlAIM1* plants, suggesting that *SlSOS1* either acts upstream of *SlAIM1* or is independent of *SlAIM1*. Thus, *SlAIM1* appears to regulate ion homeostasis through an uncharacterized pathway.

Discussion

We have identified a genetic regulator, *SlAIM1*, which integrates pathogen and abiotic stress responses. The function of tomato *SlAIM1* in pathogen and abiotic stress responses has been determined. First, tomato plants with reduced expression of *SlAIM1* generated through RNAi or VIGS result in an increased susceptibility to *B. cinerea* infection, suggesting that *SlAIM1* is required for tomato resistance to *B. cinerea*. Second, *SlAIM1* RNAi plants are sensitive to increased salinity and oxidative stress, but are insensitive to ABA. Interestingly, the overexpression of *SlAIM1* was sufficient for the increased tolerance to high salinity and oxidative stress, but was not sufficient for increased *B. cinerea* resistance. *SlAIM1* also regulates Na^+ homeostasis, as revealed from the increased Na^+ accumulation in the *SlAIM1* RNAi, and the reduced accumulation in the 35S:*SlAIM1* plants. Thus, *SlAIM1* is required for tomato pathogen and abiotic stress responses, possibly by contributing to ABA signaling.

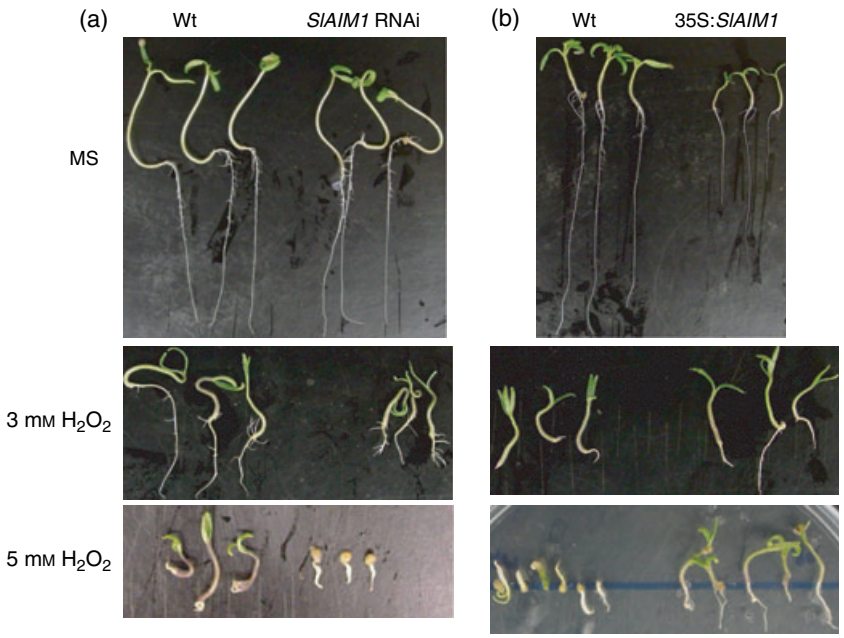

Figure 7. Tomato 35S:*SlAIM1* confers tolerance to oxidative stress generated by hydrogen peroxide (H_2O_2).
Responses of (a) *SlAIM1* RNAi and (b) 35S:*SlAIM1* plants to H_2O_2.
The seedlings shown in (a) are representative of *SlAIM1* RNAi lines 1 and 8. The seedlings in (b) are representative of 35S:*SlAIM1* lines 4 and 7. Pictures were taken 6 days after treatment with different concentrations of H_2O_2.

The specific molecular function of *SlAIM1* is still unknown. The predicted protein sequence of SlAIM1 and its nuclear localization is consistent with a DNA-binding function, and its role as a disease and ionic/oxidative stress tolerance factor has clearly been established with this study. The *SlAIM1* RNAi plants are susceptible to *B. cinerea*, and are sensitive to salt and oxidative stress, which is accompanied by a reduced sensitivity to ABA. The altered pathogen and stress responses may result from the impaired ABA-dependent activation of SlAIM1-controlled protective mechanisms. Consistent with this, the *SlAIM1* transcript accumulates in response to the exogenous application of ABA, pathogens, and salt and oxidative stress. These data suggest that SlAIM1 regulates the crosstalk between biotic and abiotic stress responses in tomato. The tomato mutant *tos1*, affected in ABA signaling, shows hypersensitivity to osmotic and salt stresses, but is insensitive to exogenous ABA (Borsani *et al.*, 2002). The tomato *TOS1* gene has not been identified, and the *B. cinerea* resistance of the mutant was not determined, but its ABA and abiotic stress responses suggest a similarity with the function of SlAIM1.

SlAIM1 shares high sequence identity with the Arabidopsis BOS1, MYB78 and MYB2 in the R2 and R3 MYB DNA-binding domains. The C-terminal region of SlAIM1 is unique, and shares no significant sequence similarities with the related MYB proteins. Blast searches against the Arabidopsis genome using SlAIM1 return Arabidopsis MYB78 and BOS1, and with the highest scores. However, because the whole genome sequence of tomato is not yet available, we cannot exclude the possibility that another tomato gene more closely related to AtBOS1 or AtMYB78 exists. SlAIM1 and BOS1 are both induced by *B. cinerea* and other stress factors, and are required for resistance to *B. cinerea*, salt and oxidative stresses. Among the closely related MYBs, *myb78* and *myb112* mutants show wild-type levels of *B. cinerea* and *A. brassicicola* resistance. This, coupled with the phenotypes of Arabidopsis *bos1* and tomato *SlAIM1* RNAi plants, suggest SlAIM1 is the functional homolog of Arabidopsis BOS1. Interestingly, Arabidopsis MYB2 has been implicated in osmotic, ABA and drought tolerance (Abe *et al.*, 2003). The ectopic expression of MYB2 causes reduced plant growth, similar to *35S:SlAIM1.* MYB2 shows significant sequence similarity with SlAIM1 around the conserved domains, but, overall, it is divergent from the other BOS1-related MYBs. Expression of *C. plantagineum* CPM10 in Arabidopsis increased salt tolerance (Villalobos *et al.*, 2004). Thus, SlAIM1, CPM10, Arabidopsis BOS1 and MYB2 perform similar functions.

Arabidopsis BOS1 and tomato AIM1 also show differences in the extent of their disease resistance functions, regulatory mechanisms and the genetic requirements for their expression. The *B. cinerea*-induced expression of *BOS1* requires functional JA responses (Mengiste *et al.*, 2003), whereas the *SlAIM1* expression was unaffected by the tomato mutation *jai1* (*coi1*) and other JA response mutants. The autoactivation of BOS1 in yeast two-hybrid assays, but the lack of autoactivation in SlAIM1 and the complete divergence of the C-terminal sequences, may suggest significant differences in their regulatory functions. *SlAIM1* is not required for responses to *P. syringae*, whereas Arabidopsis *bos1* shows increased disease symptoms after inoculation with *P. syringae* (Mengiste *et al.*, 2003).

The plant stress hormone ABA has been recognized as a regulator of disease resistance through its interactions with other defense-mediating hormones. In tomato, the lack of ABA synthesis increases resistance to *B. cinerea* as a result

of increased SA-regulated defense gene expression, faster accumulation of H_2O_2 and the associated cell wall modifications (Audenaert *et al.*, 2002; Asselbergh *et al.*, 2007). The Arabidopsis ABA-insensitive *abi1-1* and *abi2-1* mutants show an increased susceptibility to the necrotrophic bacterial pathogen *R. solanacearum*, but show resistance to the necrotrophic fungus *Plectospharella cucumerina* (Hernandez-Blanco *et al.*, 2007). The ABA insensitivity and *B. cinerea* susceptibility of *SlAIM1* RNAi plants is similar to the role of ABA in resistance to *R. solanacearum*, *P. irregular* and *A. brassicicola* in Arabidopsis (Adie *et al.*, 2007a; Hernandez-Blanco *et al.*, 2007), but contradicts some of the observations showing ABA as a negative regulator of disease resistance (Anderson *et al.*, 2004). Thus, ABA is either required for resistance or suppresses resistance depending on the specific pathogen involved, rather than whether the pathogen is a necrotroph or biotroph. ABA also mediates responses to ROIs, a common factor in pathogen and abiotic stress (Laloi *et al.*, 2004). The Arabidopsis NADPH-dependent respiratory burst oxidase homolog genes, *AtrbohD* and *AtrbohF*, are required for reactive oxygyen species (ROS) generation, ABA-induced stomatal closure and the hypersensitive response to avirulent pathogens (Torres *et al.*, 2002; Kwak *et al.*, 2003). Impaired *SlAIM1* function causes impaired ABA signaling and enhances the susceptibility to *B. cinerea*, suggesting a positive role for ABA in resistance *B. cinerea*.

Intriguingly, the ectopic expression of *SlAIM1*, although conferring resistance to oxidative stress and salinity, failed to increase resistance to *B. cinerea*, indicative of a complex mechanism of plant resistance to *B. cinerea*, consistent with the multiplicity of *Botrytis* virulence factors, including CWDEs, ROIs and toxins. Interestingly, the increased resistance to H_2O_2 and salinity, but not to *B. cinerea*, in the *SlAIM1* overexpression tomato lines suggests that oxidative stress caused by *B. cinerea* during infection may not be the critical factor in disease development. *B. cinerea* strains impaired in the generation of ROS show reduced virulence (Edlich *et al.*, 1989). In other plants, *B. cinerea* is known to cause an oxidative environment and contributes to disease development (Muckenschnabel *et al.*, 2002), and the scavenging of ROIs was suggested as a resistance mechanism (Elad, 1992).

Expression of the *SlAIM1* gene is tightly regulated, with an undetectable level of basal expression. High ectopic expression of *SlAIM1* results in reduced plant growth compared with control plants under normal growth conditions. However, under conditions of high salinity, the 35S:*SlAIM1* plants grow significantly more than the wild-type controls, and only marginally lower than the 35S:*SlAIM1* plants grown without salt stress. These data suggest that SlAIM1 controls an energy-demanding protective mechanism in the 35S:*SlAIM1* plants, which in the absence of stress, confers a growth disadvantage. In Arabidopsis, various genes are required for salt tolerance, and some of these were sufficient for tolerance when overexpressed (Shi *et al.*, 2003). In the case of tomato, although salt-sensitive mutants were identified, no genes were identified. Transgenic tomato plants overexpressing the Arabidopsis *NHX1* gene, encoding the vacuolar Na^+/H^+ antiporter, were tolerant to high levels of salinity (Zhang and Blumwald, 2001). SlAIM1 differs from these proteins, and is a transcription factor that is likely to have a regulatory role in the activation of defense against pathogens and abiotic stress, including the regulation of antiporters and ion channels. Transmembrane ion fluxes represent important early stages in pathogen-induced necrosis or HR cell death (Hahlbrock *et al.*, 1995). The involvement of ion channels in defense has been established by genetic data from Arabidopsis *dnd1* and *hlm1* mutants, which have altered disease responses that are caused by perturbations in the cyclic nucleotide-gated ion channels (CNGCs) (Clough *et al.*, 2000; Balague *et al.*, 2003). Arabidopsis HLM1 (CNGC4) is permeable to both K^+ and Na^+. Interestingly, the Arabidopsis *dnd1* mutant impaired in CNGC2 shows an increased resistance to *B. cinerea* (Govrin and Levine, 2000). The necrosis and tissue collapse that occurs in *SlAIM1* RNAi plants in response to *Botrytis* and high salinity could result from unregulated ion fluxes early in infection, or exposure to abiotic stress.

In conclusion, SlAIM1 regulates pathogen and abiotic stress responses by modulating events common to biotic and abiotc stress. SlAIM1 may be required for ABA signaling, which in turn controls protective mechanisms. Future studies should focus on the molecular functions of SlAIM1 and potential targets that effect tomato responses to pathogen and stress signals.

Experimental procedures

Plant growth

Tomato cultivars CastlemartII and Micro-Tom were grown in plastic pots containing compost soil mix in a glasshouse, with a photoperiod that was extended to 15 h under fluorescent lights (160 $\mu mol^{-1}\ m^{-2}\ s^{-1}$) at a day/night temperature of 22/18°C $\pm$ 4°C. The tomato mutants *spr1*, *spr2*, *def1*, *acx1*, *jai1* and *SlAIM1* RNAi plants are in the CastlemartII background. All plants were fertilized twice weekly before any biotic or abiotic stress experiment.

Fungal and bacterial disease assays

The culture of the *B. cinerea* strain *BO5-10* used for the disease assays and the preparation of conidial spore suspension were described previously (Mengiste *et al.*, 2003). Tomato and Arabidopsis disease assays and determination of fungal growth were performed as described by AbuQamar *et al.* (2008). Bacterial disease assays were carried out using the standard leaf infiltration protocol, essentially as described by Mengiste *et al.* (2003), spray inoculation (Zipfel *et al.*, 2004) or seedling incubation (Uppalapati *et al.*, 2008). Leaves of 6-week-old tomato plants were infiltrated

with suspension (OD_{600} = 0.001 in 10 mM $MgCl_2$) of the bacterial strain *P. syringae* pv. *tomato* DC3000 (a generous gift of Greg Martin, Cornell University). To determine bacterial growth, leaf discs from infected leaves were collected at 0, 2 and 4 dpi. Each experiment for the bacterial growth assay was performed in three replicates. At each time point, two leaf discs were collected from wild-type and *SlAIM1* RNAi plants for each replicate. Leaf discs of the same size were made using a hole puncher, and the bacterial titer per leaf area was determined. In parallel, plants were inoculated by spraying with bacterial suspension. Bacterial cultures grown overnight were collected, washed once and resuspended in sterile water containing 0.04% Silwet L-77 (Lehle Seeds, http://www.arabidopsis.com), and the solution was then sprayed on plants. Leaves from the spray-inoculated plants were harvested and surface sterilized (30 sec in 70% ethanol, followed by 30 sec in sterile distilled water), and were then used to determine bacterial growth. In addition, tomato seedlings were assayed for bacterial responses, as recently described (Uppalapati *et al.*, 2008). Seedlings (5-days old) containing 2–3-cm-long hypocotyls that were germinated under axenic conditions were inoculated by flooding MS agar plates with a bacterial suspension, until the seedlings were completely submerged (OD_{600} = 0.1). The seedlings were exposed to bacterial suspension for 2–3 min with gentle mixing. The bacterial suspension was then discarded, inoculated seedlings were incubated in a growth room with a 12-h photoperiod and the disease responses were observed for up to 7 days after inoculation.

RT-PCR

For RT-PCR, cDNA was synthesized from both control and treated samples using equal quantities of total RNA (2 μg), AMV reverse transcriptase (Promega, http://www.promega.com) and oligo (dT_{15}) primers, according to standard protocols. The PCR was performed for 35 cycles using 2.5 μl of cDNA as a template and specific primer pairs (94°C 30 sec, 52°C 30 sec, 72°C 1 min). The amplified products were separated on 1.5% agarose gels and visualized under UV light after staining with ethidium bromide. The *SlAIM1* (forward, 5′-CTCGTTGGGGCAATAGGTGGTCAAA-3′; reverse, 5′-CGTTACACTAGAAAATTCCCGCGTGG-3′) primers were used for RT-PCR. The tomato *translation initiation factor* (*eIF4A*) gene was amplified as a control to demonstrate the relative quantity of cDNA. The other primer sequences used for RT-PCR were recently described by AbuQamar *et al.* (2008).

Hormone and wounding treatments

Six-week-old tomato seedlings were used to confirm the *SlAIM1* expression in response to abiotic stresses. For hormone and paraquat treatments, a concentration of 100 μM of paraquat (methyl viologen), ABA, MeJA, ACC or SA was sprayed. For the salt treatment, a concentration of 200 mM NaCl was applied. Mechanical wounding was performed by wounding the main veins of apical leaflets of compound leaves with dented forceps.

DNA and RNA blots

For DNA blots, genomic DNA was extracted from wild-type tomato leaves as described by Dellaporta *et al.* (1983). A 10-μg genomic DNA was digested with restriction enzymes and subsequently separated on a 0.8% (w/v) agarose gel. Total RNA from tomato leaf tissues was extracted from tissues frozen in liquid nitrogen, as described by Lagrimini *et al.* (1987). RNA was separated on 1.2% formaldehyde agarose gels. The gels were then blotted onto Hybond N^+ nylon membranes (Amersham Pharmacia Biotech, now part of GE Healthcare, http://www.gelifesciences.com). Probes were labeled with ^{32}P by random priming using a commercial kit (Sigma-Aldrich, http://www.sigmaaldrich.com). The hybridization of the probe and subsequent washings were performed as described by Church and Gilbert (1984).

Rapid amplification of cDNA ends (RACE)

The RACE experiments were performed by using the BD SMART™ RACE cDNA Amplification Kit (cat. no. 634914; BD Biosciences, http://www.bdbiosciences.com). The remaining steps and modifications were described previously by AbuQamar *et al.* (2008).

Vector construction

To make 35S:*SlAIM1* overexpression constructs, the *SlAIM1* full-length cDNA was amplified by PCR from the RACE-Ready cDNA with primers SlAIM1/LP (5′-TCCCCGCGGATGGATAAATTAATCAATCAAGAA-3′, with the *Sac*I site underlined) and SlAIM1/RP (5′-CGCGGATCCTAATTAGGACCAAATGTCTTCAAT-3′, with the *Bam*HI site underlined). Restriction-digested PCR products were cloned into the pCAMBIA 99-1 vector (a modified version of the binary vector pCAMBIA 1200) behind the double cauliflower mosaic virus (CaMV) 35*S* promoters between the *Sac*I and *Bam*HI sites.

To generate the *SlAIM1* RNAi construct, 250 bp from the 3′ end of the cDNA, including part the 3′ untranslated region (3′-UTR), of *SlAIM1* were amplified by PCR from the RACE-Ready cDNA, with primers SlAIM1 RNAi/LP (5′-GCACTAGTCCATGGCGAATTATAATTGTATTAA-3′, with the *Spe*I and *Nco*I sites underlined), and SlAIM1 RNAi/RP (5′-CGGGATCCGGCGCGCCGGACCAAATGTCTTCAAT-3′, with the *Bam*HI and *Asc*I sites underlined). The inverted repeat is assembled directly in the binary vector by a two-step cloning process using the introduced restriction enzyme sites, as described by AbuQamar *et al.* (2008).

Plant transformation and regeneration, and virus-induced gene silencing

Tomato transformation was carried out as described by Howe *et al.* (1996), and the specific modifications and details for the tomato transformation and regeneration were described in detail by AbuQamar *et al.* (2008). The *SlAIM1* silencing was performed using the TRV vector system, as described by Liu *et al.* (2002).

Salt stress experiment

For experiments in tissue culture, tomato seeds were surface-sterilized with 35% (v/v) commercial bleach for 30 min, and were then washed several times with sterile water. For the germination experiments, seeds were germinated on MS medium with 3% (w/v) sucrose and 0.7% (w/v) agar, containing 0, 50, 100 and 150 mM sodium chloride (NaCl). For the root growth experiment, between 30 and 40 seeds from each genotype were germinated on basal MS medium. Seedlings (4-days old) with ~2-cm-long roots were transferred from vertical basal MS plates onto other plates of MS medium, containing 125 mM NaCl (Borsani *et al.*, 2002). For root length growth measurements, the root lengths of 10 seedlings were measured per treatment, and three replicates were run for each treatment. Changes in root length were measured after 2 days of treatment.

For glasshouse experiments, seeds were sown in D40 deepots (Stuewe & Sons, Inc., http://www.stuewe.com) containing turface

calcined clay (Profile Products, http://www.profileproducts.com). Deepots were placed in a greenhouse with a photoperiod extended to 15 h using incandescent and fluorescent lights (160 μmole m^{-2} s^{-1}) at a day/night temperature of 26/18°C ± 4°C. Seeds were mist-watered for 16 sec every 10 min. After the seedlings germinated, deepots within blocks were randomized. Plants were subirrigated as needed with purified water containing 0 mM NaCl. After 4 days, plants were subirrigated for 2 h daily with fertilizer solution that contained 0, 50, 100, 150, 200 or 250 mM NaCl. The fertilizer solution contained (in mg per liter) 200 N, 29 P, 167 K, 67 Ca, 30 Mg and micronutrients supplied from a commercial fertilizer formulation (Miracle Gro® Excel® 15-5-15 Cal-Mag; The Scotts Co., http://www.scotts.com). Three-week-old seedlings of the Castlemartll wild-type cultivar and *SlAIM1* RNAi line, or the 5-week-old Micro-Tom wild-type cultivar and the 35S:*SlAIM1* line, were harvested. Shoot and root tissues were collected separately, dried at 65°C and weighed.

Tissue Na^+ quantification

Leaf tissue (5 ± 1.5 mg dry weight) was sampled into Pyrex tubes (16 × 100 mM) and dried at 92°C for 20 h. After cooling, five samples per replicate per treatment from each genotype were weighed, digested and elemental analysis was performed with an ICP-MS for Li, B, Na, Mg, P, K, Ca, Mn, Fe, Co, Ni, Cu, Zn, As, Se, Mo and Cd, as described by Rus *et al.* (2006).

Phylogenetic analysis

The conserved regions in the SlAIM1 and related R2R3 MYBs were used for constructing the SlAIM1 phylogenetic tree. Sequences were aligned using CLUSTALW (Thompson *et al.*, 1994) with default gap penalties, and the alignment was manually adjusted where necessary. Mean character distances were used to construct the unrooted neighbor-joining phylogeny (Saitou and Nei, 1987) from the PHYLP v3.67 package (Felsenstein, 1993). Statistical support of the branches was tested with 1000 bootstrap resamples.

Data deposition

DNA sequences of the tomato AIM1 and the predicted amino acid sequences have been deposited in GenBank under accession no. EU934734.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Figure S1. Sequence comparison and phylogenetic relationship between tomato AIM1 and related MYB proteins.
Figure S2. *SlAIM1*-related Arabidopsis MYBs, *MYB112* and *MYB78*, have no resistance function against infection by necrotrophic fungi.
Figure S3. Virus-induced gene silencing of *SlAIM1* in tomato plants (*SlAIM1* VIGS) causes an increased susceptibility to *Botrytis cinerea.*
Figure S4. The overexpression of *SlAIM1* was not sufficient to confer an increased resistance to *Botrytis cinerea* in tomato.
Figure S5. *SlAIM1* is required for germination and growth on salt.
Figure S6. Responses of wild-type tomato and *SlAIM1* RNAi plants at different time points after exposure to salt stress.
Figure S7. Root growth in 35S:*SlAIM1* plants exposed to different salt concentrations at 33 days after NaCl treatment.
Figure S8. Growth of 35S:*SlAIM1* plants grown under continuous salt treatment.
Figure S9. Comparisons of the concentrations of various elements in tomato *SlAIM1* RNAi and wild-type plants.
Figure S10. Comparisons of the concentrations of various elements in tomato 35S:*SlAIM1* and wild-type plants.
Table S1. *R2R3MYB* genes closely related to *SlAIM1*, and their expression in response to pathogens, plant hormones and other stresses.
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