

RESEARCH PAPER

Multiple phytohormone signalling pathways modulate susceptibility of tomato plants to *Alternaria alternata* f. sp. *lycopersici*

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Abstract

Three phytohormone molecules – ethylene (ET), jasmonic acid (JA) and salicylic acid (SA) – play key roles in mediating disease response to necrotrophic fungal pathogens. This study investigated the roles of the ET, JA, and SA pathways as well as their crosstalk during the interaction between tomato (*Solanum lycopersicum*) plants and a necrotrophic fungal pathogen *Alternaria alternata* f. sp. *lycopersici* (AAL). Both the ET and JASMONIC ACID INSENSITIVE1 (JAI1) receptor-dependent JA signalling pathways are necessary for susceptibility, while SA response promotes resistance to AAL infection. In addition, the role of JA in susceptibility to AAL is partly dependent on ET biosynthesis and perception, while the SA pathway enhances resistance to AAL and antagonizes the ET response. Based on these results, it is proposed that ET, JA, and SA each on their own can influence the susceptibility of tomato to AAL. Furthermore, the functions of JA and SA in susceptibility to the pathogen are correlated with the enhanced or decreased action of ET, respectively. This study has revealed the functional relationship among the three key hormone pathways in tomato defence against AAL.

Key words: AAL, ethylene, hormonal interactions, jasmonic acid, salicylic acid, tomato.

Introduction

Plants have evolved complex defence mechanisms to defend themselves against different pathogens, which are encountered throughout their lifetimes (Nürnberg and Lipka, 2005). Pathogen attack triggers complex signalling cascades regulated by signalling molecules such as jasmonic acid (JA), salicylic acid (SA), and ethylene (ET), resulting in plant defence responses (Sheard *et al.*, 2010). These defence responses are considered to be dependent on specific plant–attacker interactions. In general, SA-mediated signalling pathways are implicated in resistance to biotrophs or hemibiotrophic pathogens, whereas JA- and ET-dependent responses are effectively against necrotrophs and herbivorous insects (McDowell and Dangel, 2000). For example,

Arabidopsis mutants that are impaired in SA production, as well as *NahG* transgenic plants lacking SA, exhibit enhanced disease susceptibility to a variety of biotrophic pathogens, including the fungal pathogen *Hyaloperonospora arabidopsidis* and the bacterial pathogen *Pseudomonas syringae* (Kunkel and Brooks, 2002). In contrast, the JA-perception mutant *coronatine insensitive 1 (coi1)* shows severely compromised resistance to the necrotrophic fungal pathogens *Alternaria brassicicola* and *Botrytis cinerea* and the bacterial leaf pathogen *Erwinia carotovora* (Thomma *et al.*, 1998).

Plant hormones usually do not regulate defence responses via a linear mode of action but through complex network of

regulatory interactions. One of the best-studied examples is the interaction between the SA and JA signalling pathways (Kunkel and Brooks, 2002; Koornneef and Pieterse, 2008). For example, exogenous SA application inhibits JA-induced proteinase inhibitor gene expression and protein accumulation in tomato (*Solanum lycopersicum*) (Doares *et al.*, 1995), whereas methyl jasmonate (MeJA) treatment inhibits SA-induced acidic pathogenesis-related (PR) gene expression in tobacco (Niki *et al.*, 1998). Reduced susceptibility to the coronatine-producing strains of *P. syringae* in *Arabidopsis* mutants *coil* and *jin1*, which are defective in JA signalling, is correlated with elevated PR1 expression and is dependent on SA accumulation (Kloek *et al.*, 2001; Laurie-Berry *et al.*, 2006). Coronatine promotes susceptibility by stimulating JA signalling and inhibiting SA-mediated defence that normally limits the growth of *P. syringae* (Kunkel and Brooks, 2002; Laurie-Berry *et al.*, 2006). Infection with *P. syringae*, which induces SA-mediated defence, renders plants more susceptible to the necrotrophic pathogen *Alternaria brassicicola* by suppression of the JA signalling pathway (Spoel *et al.*, 2007). The above pharmacological and genetic evidence shows that the crosstalk between SA and JA is antagonistic. However, cooperation and sequential positive interactions have also been reported between the SA and JA pathways (Mur *et al.*, 2006). JA and ET are considered to act synergistically in response to pathogens and activate defence-related gene expression in *Arabidopsis* (Thomma *et al.*, 1999). Microarray data has revealed that nearly half of the genes induced by ET are also induced by JA (Schenk *et al.*, 2000). Lorenzo *et al.* (2003) reported that JA and ET pathways converge in the transcriptional activation of ETHYLENE RESPONSE FACTOR1 (ERF1), which encodes a transcription factor that regulates the expression of pathogen response genes.

Most knowledge about crosstalk in plant hormone signalling is from *Arabidopsis*, mainly due to the availability of genetic and genomic resources. It will be interesting to investigate the response of plants to diverse pathogens in other plant species. Tomato is not only a major vegetable crop, but also a useful model plant for studying the complex network of hormone pathways involved in host defence response, for many mutants or transgenic plants that are altered in hormonal metabolism or signalling have been characterized. In tomato, damage to a single leaflet by mechanical wounding or an insect results in production of systemin, an 18-amino-acid peptide (Pearce *et al.*, 1991). Interaction of systemin with its proposed receptor triggers the activation of the octadecanoid pathway for JA biosynthesis, which induces defence gene expression (Sun *et al.*, 2011). JA-deficient mutants *def1* (*defenseless-1*, with a defective octadecanoid synthesis pathway) and *spr2* (a suppressor of (*pro*)systemin-mediated responses2 mutation with reduced levels of trienoic fatty acids) have been characterized (Li *et al.*, 2003). In addition, the *35S::prosystemin* transgenic line (*35S::prosys*), which overexpresses prosystemin, constitutively accumulates high levels of proteinase inhibitor proteins throughout the entire plant (Howe and Ryan, 1999). The F-box protein CORONATINE INSENSITIVE 1 (COI1) is a key player in the JA pathway (Sheard *et al.*, 2010). A JA-insensitive mutant *jail* (*jasmonic acid insensitive1*) contains a mutation in the tomato homologue of *Arabidopsis*

COI1 and fails to express JA-regulated genes in response to wounding and MeJA (Li *et al.*, 2004). Moreover, several ET mutants in tomato, such as *Nr* (*Never ripe*) and *epi* (*epinastic*), have been characterized. *Nr* is an ET-insensitive mutant, caused by a single base substitution in the N-terminal coding region of the ET receptor gene *ETR3* (i.e. *NR*) that is homologous to *ETR1* in *Arabidopsis* (Wilkinson *et al.*, 1995). The ET-overproducing mutant *epi* is constitutively activated in a subset of ET responses (Fujino *et al.*, 1988). To study SA responses, transgenic *NahG* plants expressing the enzyme salicylate hydroxylase were used. The enzyme converts endogenous SA immediately to inactive catechol, therefore the plants are deficient in SA accumulation (Brading *et al.*, 2000).

The necrotrophic pathogen *Alternaria alternata* f. sp. *lycopersici* (AAL) causes *Alternaria* stem canker on some susceptible tomato cultivars. The disease is characterized by dark-brown canker formation on stems and leaf tissue necrosis between the veins (Brandwagt *et al.*, 2002). The fungus produces a group of mycotoxins, AAL toxins, which have been shown as a host-specific pathogenicity factor (Brandwagt *et al.*, 2002). AAL toxins, together with another group of structurally related mycotoxins, fumonisins, are classified as sphinganine-analogue mycotoxins due to their structural similarity to sphinganine (Brandwagt *et al.*, 2002). Resistance to AAL or insensitivity to AAL toxins in tomato is determined by the single co-dominant *Alternaria Stem Canker* (*ASC*) locus. The *Asc-1* gene is homologous to the yeast *Longevity Assurance Gene 1* (*LAG1*) (Brandwagt *et al.*, 2002). Using a protoplast model system, Asai *et al.* (2000) showed that fumonisin B₁-induced cell death in *Arabidopsis* requires the JA-, SA-, and ET-mediated signalling pathways, but not the signal transmitter NPR1. Previous reports indicated that an ET-dependent pathway is involved in AAL-toxin-induced cell death (Moore *et al.*, 1999; Asai *et al.*, 2000). Recently, Zhang *et al.* (2011) studied the role of JA and ET in AAL-toxin-induced tomato cell death and showed that both JA and ET promote AAL-toxin-induced cell death alone and that JASMONIC ACID INSENSITIVE1 (JAI1) receptor-dependent JA signalling promotes programmed cell death through ET action. Egusa *et al.* (2009) also reported that JA promotes the susceptibility of detached tomato leaves to AAL infection. However, the role of plant hormones and their crosstalk in the interaction between tomato plants and AAL remain to be investigated.

The present study, using ET, JA, and SA mutants combined with exogenous application, demonstrates that both the ET response and the JA-dependent signalling pathway are necessary for susceptibility of tomato to AAL fungus, while the SA pathway is responsible for the resistance to this pathogen. Furthermore, it is observed that the role of JA and SA in susceptibility to the pathogen is associated with the action of ET the pathway. The significance of these findings is also discussed.

Materials and methods

Plant materials and growth conditions

Tomato cultivar Castlemart (CM) is the parental line of JA mutants *spr2* and *jail* as well as the transgenic line *35S::prosys*. Homozygous

jail seedlings were selected from F2 populations as described previously (Li *et al.*, 2004). *35S::prosys* seeds were collected from a *35S::prosys* homozygote (Howe and Ryan, 1999) that had been backcrossed five times to its wild-type (WT) line cv. CM as the recurrent parent (Li *et al.*, 2002). The ET-overproducing mutant *epinastic* (*epi*), which is constitutively activated in a subset of ET responses (Fujino *et al.*, 1988), and its WT line, VFN8, were obtained from the Tomato Genetics Resource Center (University of California, Davis, CA, USA). Tomato seeds of *NahG* transgenic line and its WT cv. Moneymaker (MM) were kindly provided by Dr. Jonathan D. G. Jones (John Innes Centre, Norwich, UK).

Seeds were sown in seedling trays containing a rich soil mixture after germination on filter paper. Seedlings were grown in a greenhouse, with temperature ranging from 22 to 28 °C (night and day air temperature, respectively) and a 16/6 light/dark cycle. Three weeks after germination, seedlings were transplanted to plastic pots (12 cm diameter, 15 cm depth) filled with perlite and turfy soil (3:1, v/v), which were watered daily and fertilized weekly with a half-strength Enshi nutrient solution (Zhang *et al.*, 2011). Tomato plants of 7 weeks of age were used in this study.

Fungal infection assay

Fungal culture and infection were performed as described by Brandwagt *et al.* (2002). In brief, the AAL isolates were grown for 14 days on corn meal agar (CMA) at 25 °C in the dark. Spores were washed by water, filtered, and washed twice in tap water to remove partial in-vitro-produced AAL toxins. The spore quantity was counted using a haemocytometer. Tomato plants of 7 weeks of age were sprayed with spore suspensions containing approximately 1.0×10^6 spores ml⁻¹ and 0.1% (v/v) Tween 20 and then covered with plastic domes to keep high humidity at 24–26 °C for 48 h. Then, the domes were removed and disease symptoms emerged 3–4 days after infection.

Chemical pretreatments

Tomato plants were subjected to different chemical pretreatments before AAL inoculation. Solutions of the ET-action inhibitor silver thiosulphate were prepared by mixing AgNO₃ and sodium thiosulphate at a concentration ratio of 1:4 (Bellés *et al.*, 1993). Solutions of the ET-biosynthesis inhibitor CoCl₂ were prepared by dissolving in ultrapure water. 1-Aminocyclopropane-1-carboxylic acid (ACC, Sigma, St Louis, MO, USA) was prepared by dissolving in ultrapure water. Solutions containing 0.1 mM ACC, 1 mM silver thiosulphate, and 0.1 mM CoCl₂ (all containing 0.1% Tween 20) were applied to plants 24 h before AAL infection, while controls were treated with equal amount of 0.1% Tween 20.

1-Methylcyclopropane (1-MCP) treatment was performed as described previously (Diaz *et al.*, 2002). AAL infection was conducted after 48 h of 1-MCP treatment.

SA was dissolved in absolute ethanol then added drop wise to 20 mM phosphate buffer solution (pH 7.0) (ethanol/phosphate buffer, 1:1000, v/v). For SA pretreatment, tomato seedlings were soil-drenched with 50 ml of 0.2, 1, or 2 mM SA, respectively, and 20 mM phosphate buffer solution (pH 7.0) was used as a control.

For methyl jasmonate (MeJA) treatment, plants were transferred into a lucite container (84 L) containing 18 µl MeJA (Sigma). Six cotton wicks were distributed evenly throughout the box and each containing 300 µl MeJA solution (3 µl MeJA, diluted into 297 µl ethanol). Twenty-four hours after MeJA treatment, the cotton wicks were removed and the plants were acclimated to ambient humidity for an additional 24 h before AAL infection. Control plants were incubated in a separate container in which ethanol was applied to cotton wicks.

Preparation of endogenous ET-deficient plants and ET-insensitive plants

Tomato WT cv. CM seedlings were divided equally into three groups and sprayed with different solutions to the whole plants weekly from

the cotyledon stage to the five-leaf stage. The first group was sprayed with distilled water and used as a control; the second and the third groups were sprayed with 0.1 mM CoCl₂ and 1 mM silver thiosulphate, respectively. The plants in the second and the third groups were used as endogenous ET-deficient and ET-insensitive plants, respectively.

Real-time quantitative PCR (qPCR) analysis

Tomato leaves (at least five leaves from independent plants) were harvested following AAL infection at different time points (0, 1, 3, and 5 d) and immediately immersed in liquid nitrogen. Total RNA was isolated using TRIzol reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instructions, and then treated with RNA free DNase I (Takara, Japan) to remove genomic DNA contamination. For reverse transcription, oligo(dT)18 primers, 10 mM dNTP mix, and water were added to 5 µg RNA, following the manufacturer's protocol (Fermentas, Canada). Quantitative PCR was performed as described previously (Zhang *et al.*, 2011). All experiments were repeated twice, with similar results. For each experiment, data were analysed separately. Results of one representative experiment are shown. The primer sequences used are listed in Table 1.

Fungal biomass measurement

Fungal biomass was measured according to Egusa *et al.* (2009) with minor modifications. Total genomic DNA was extracted from 1.0 g tomato leaves (from independent plants) at 6 d post inoculation using 2 % CTAB method. Extracted DNA was diluted 1/100 and used for qPCR template. The primer pairs (DeH-F, 5'-CTCCGCCTGCCAATGTGATTAC-3' and E8T7, 5'-GCGTACCAAGGCACGTGCTCAA-3') designed for the amplification of the gene for AAL toxin biosynthesis (*ALTI*) were used for detecting the tomato pathotype of AAL (Egusa *et al.*, 2009).

Ethylene quantification

Ethylene was quantified with a gas chromatograph (HP5890, Agilent Technologies, USA) as described previously (Lund *et al.*, 1998).

Statistical analysis

Data were analysed using Statistica (SAS Institute, <http://www.statsoft.com>). Differences in expanding lesions per plant at each time point in each figure (except for Fig. 3A) and the differences in relative fungal DNA in each figure were analysed by one-way analysis of variance (ANOVA). If the ANOVA analysis was significant ($P < 0.05$), Duncan's multiple range test was used to detect significant differences between groups. In Fig. 3A and Supplementary Fig. S2, the differences in expanding lesions per plant at each time point were analysed by Student's t-test.

Results

ET modulates tomato susceptibility to AAL

Previous reports revealed that ET is involved in AAL-toxin-induced tomato cell death (Moore *et al.*, 1999; Zhang *et al.*, 2011). This led the present study to test the role of ET in modulating defence of tomato plants to the toxigenic AAL fungus using the ET action inhibitors silver thiosulphate and 1-MCP and the ET precursor ACC to establish the effect of reduced ET perception or increased ET production, respectively.

Typical necrotic lesions appeared on stems at approximately 3 or 4 d after AAL infection, while the foliar symptom appearance was later than that of the stem. Expanding

Table 1. Primer sequences used for real-time quantitative PCR.

Gene	GenBank accession	Forward primer (5'–3')	Reverse primer (5'–3')	Product (bp)
<i>Actin</i>	AB199316	TGGTCGGAATGGGACAGAAG	CTCAGTCAGGAGAACAGGGT	190
<i>LOXD</i>	U37840	GGCTTGCTTTACTCCTGGTC	AAATCAAAGCGCCAGTTCTT	72
<i>AOS2</i>	AF230371	CGATTACCTCCGATTCTGGT	AAATCTTCATCCCACCGAAG	172
<i>PI-II</i>	K03291	TGATGAACCCAAGGCAAATA	ACACAACCTTGATGCCACAT	154
<i>PAL</i>	M83314	ACAGAATTGTTGACGGGTGA	CCATTCCAGCTCTTCAGACA	122
<i>ICS</i>	DQ984132	TCCAGGCTGAAGATGATGAG	TTATTTCCAAACCGCAAATTC	183
<i>NPR1</i>	AY640378	GGAAACTTCACTGGCAGACGTC	GTGTCTCTGAAACTTGTGCGACC	415
<i>PR1a</i>	X71592	TGGTATGGCGTAAGTCGGTA	CTTGAATCAAAGTCCGGTT	152
<i>PR2a</i>	M80604	AGATCTTGAAGCCCTAGCCA	TGGATCAACTTCGTTTCCAA	118
<i>ACO1</i>	X58273	TTGCTCATTTCCTTTGTGGA	GGAAGCTAGCAAAGCAAACC	122
<i>ACS2</i>	X59139	ATCCACCTTGTGTTGTGACGA	TGTTTCATCGAGGATTCAGC	86
<i>ACS4</i>	X59146	AATTGCTCGGAGGTAGGATG	TTCTCTTCCATTGTGCTTG	154
<i>ERF1</i>	AY044236	ATTGGAGTTAGAAAGAGGCCAT	CTCATTGATAATGCGGCTTG	143
<i>NR</i>	U38666	GCGGTTATGTTCTGTTCT	TGTCGAGCTACATCAAAGC	194
<i>ETR4</i>	AF118843	CTGCAGATTGGAATGAATGG	ATAAGGCACCGTCAACATCA	123

lesions on stem and leaves per plant were recorded at 4, 6, and 8 d after AAL inoculation. As shown in Fig. 1A and B, fewer lesions were observed in silver thiosulphate- or 1-MCP-pretreated plants at each time point examined, while more spreading lesions were found in ACC-pretreated plants at 4 and 6 d following the fungus infection compared with control plants. Fungal biomass in the infected plants was estimated by quantifying fungal DNA with qPCR using specific primers for *ALTI*, which is involved in AAL toxin biosynthesis and pathogenicity (Egusa et al., 2009). Consistently with disease symptoms, the amount of fungal DNA was increased by ACC pretreatment and reduced by 1-MCP treatment as compared with the control (Fig. 1C), suggesting that ET plays an important role in susceptibility of tomato to AAL.

To investigate the role of ET response on the susceptibility of tomato plants to AAL in detail, an inoculation assay was conducted on a tomato ET-overproducing mutant *epinastic* (*epi*) and its WT line cv. VFN8. The results showed that small lesions appeared on the stem and leaves of *epi* at 4 d, and the lesions increased in size over time. The disease symptoms of *epi* and VFN8 plants at 7 d post inoculation are shown in Fig. 1D. The *epi* plants wilted and died at 3 weeks after infection, while no lesion was observed in VFN8 plants even after 1 month post inoculation (data not shown).

Involvement of JA signalling pathway in susceptibility of tomato to AAL

To investigate the role of JA signalling in the interaction between tomato and AAL, JA-deficient mutant *spr2*, JA-insensitive mutant *jail1*, the transgenic line *35S::prosys*, and their WT cv. CM were used for AAL inoculation experiments. Compared with CM, *jail1* and *spr2* plants showed enhanced resistance to the fungus (Fig. 2A, B) and there were decreased expression levels of *LOXD* and *AOS2* and abolished *PI-II* expression in infected *jail1* leaves (Supplementary Fig. S1, available at JXB online). However, there were more lesions on the stems and leaves of *35S::prosys* (Fig. 2A).

Pretreatment with MeJA, on the other hand, increased the number of necrotic lesions on *spr2* and CM plants, and exogenous MeJA application restored the disease symptom of *spr2* to CM level (Fig. 2B). A positive correlation was observed between fungal biomass and the percentage of expanding lesions, the fungal biomass of *spr2* and *jail1* was significantly lower, whereas the fungal biomass of *35S::prosys* was higher than that of CM plants (Fig. 2C). These results suggest that the JA signalling pathway is involved in tomato susceptibility to AAL, which is dependent on the JAI1 receptor.

SA enhances the resistance of tomato plants to AAL

SA has a significant role in the induction of host response to pathogens. The establishment of systemic acquired resistance is associated with enhanced biosynthesis of SA and activated expression of *PR* genes, which requires the function of *Arabidopsis* *NPR1* (Yu et al., 2001). Here, to assess the potential role of SA in tomato resistance to AAL, CM plants were soil-drenched with different concentrations of SA solutions. The results show that 1 mM SA pretreatment greatly decreased lesion occurrence at each time point examined, while drenching with 2 mM SA reduced disease symptom at 4 and 6 d post inoculation (Fig. 3A). Transgenic *NahG* plants exhibited more expanding lesions than the WT line MM (Fig. 3B). The amount of fungal DNA in *NahG* plant was markedly higher than that in MM (Fig. 3C).

Arabidopsis *NahG* plants enhanced susceptibility to *P. syringae* pv. *phaseolicola* strain 3121, and exogenous application of catechol, the immediate SA-degradation product of NAHG activity, caused wild-type *Arabidopsis* to lose resistance to this strain, suggesting that this susceptibility in *NahG* plants might be due to catechol production (van Wees and Glazebrook, 2003). To investigate this possibility, MM tomato plants were treated with different concentrations of catechol (0, 0.1, 0.5, 1, 2.5, 5 mM) before AAL inoculation. No significant difference in amount of expanding lesions was observed among different treatments (data not shown),

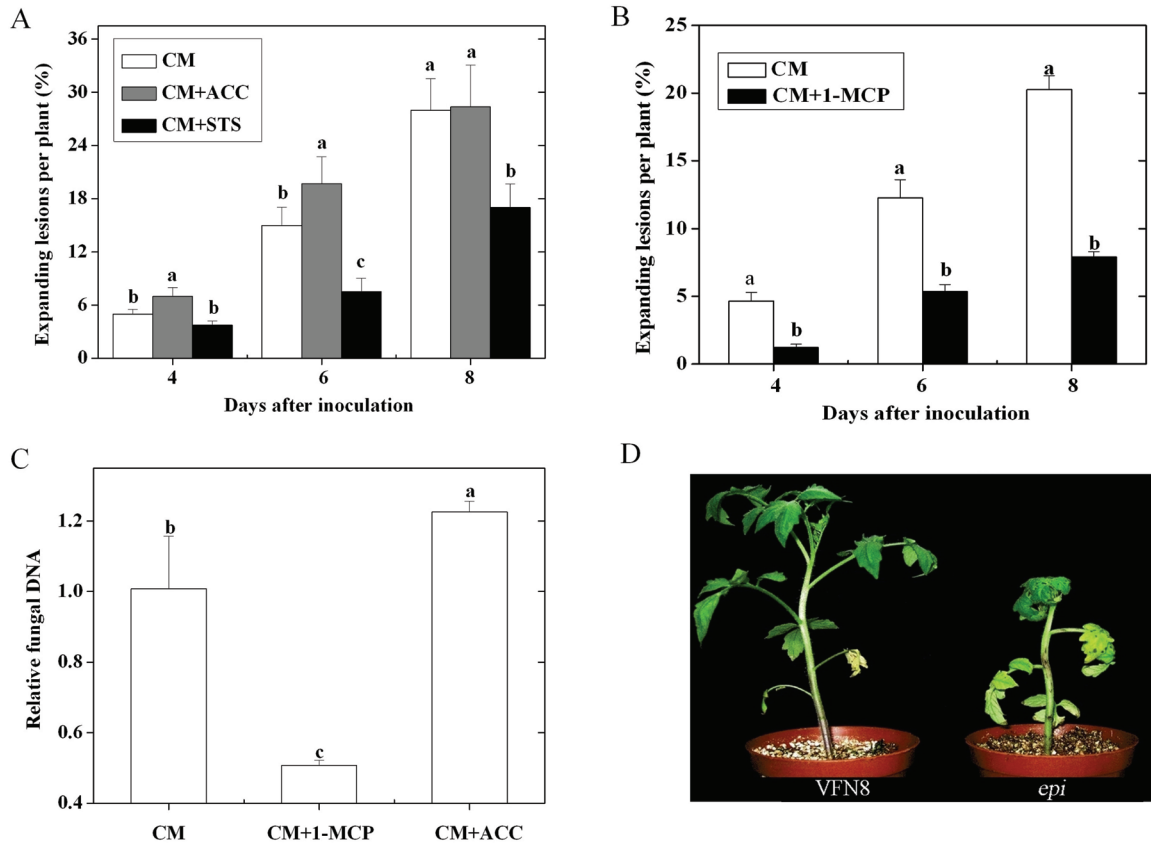


Fig. 1. Role of ET in tomato susceptibility to *A. alternata* f. sp. *lycopersici*. (A) Effect of exogenous ET precursor ACC (0.1 mM) and ET action inhibitor silver thiosulphate (1 mM) on disease development in CM plants. (B) Effect of ET receptor inhibitor MCP (10 nl l^{-1}) on the disease development in CM plants. (C) Effect of 10 nl l^{-1} 1-MCP and 0.1 mM ACC on the relative fungal DNA in CM leaves at 6 d post inoculation; the amount of fungal DNA was quantified by qPCR using *ALT1* primers. (D) Disease symptoms in *epi* and VFNS plants at 7 d post inoculation. Error bars indicate standard deviation from the mean of three replicates. For each time point in A and B, letters indicate significant differences among treatments; in C, letters indicate significant differences among treatments ($P < 0.05$, Duncan's multiple range test). The experiment was repeated three times and similar results were obtained. For each experiment, data were analysed separately. Results of one representative experiment are shown.

suggesting that catechol accumulation is not the direct cause of the enhanced susceptibility to AAL in *NahG* plants.

Several SA-dependent *PR* genes are commonly used as reporters of SA-dependent defence (Kunkel and Brooks, 2002). SA-mediated acidic *PR* gene expression in MM and *NahG* plants was also detected, the results showing that transcript levels of *PR1a* and *PR2a* in the *NahG* plant were greatly depressed compared with MM plants at 1 and 3 d post inoculation (Fig. 3D, E). The above results suggest that the SA-mediated response enhances the resistance of tomato plants to AAL infection.

Enhanced *jai1* plant resistance is correlated with decreased ET production and signalling

To determine whether the reduced disease symptom in *jai1* plants was correlated with altered ET signalling, the expression of genes encoding ET biosynthesis or signalling in *jai1* and CM leaves was profiled after AAL infection. ACC synthase (ACS) and ACC oxidase (ACO) are two key enzymes involved in ET biosynthesis (Yang and Hoffman, 1984) and

the present study detected the expression levels of *ACS4* and *ACO1*. As shown in Fig. 4A and B, the transcript levels of *ACS4* and *ACO1* were highly induced in both CM and *jai1* plants at 1 d after AAL infection. Moreover, the expression levels of both genes were markedly lower in *jai1* at 1 d in comparison with CM (Fig. 4A, B). Then, ET production was assessed in both CM and *jai1* leaves after AAL infection. Fig. 4C shows that ET emission in *jai1* was suppressed at an early stage of infection and that the ET burst in *jai1* was delayed compared with CM.

Tomato *ETR3* (*NR*) and *ETR4*, but not the other ET receptor genes, are significantly induced by pathogen infection (Wilkinson *et al.*, 1995; Klee, 2002). Transcription factor ERF1-dependent gene induction is controlled by the combined action of JA and ET in response to pathogen attack (Koornneef and Pieterse, 2008). As shown in Fig. 4D and E, the expression of *NR* and *ETR4* was strongly induced and reached a maximum at 1 d, declining but still remaining at significantly higher levels than controls at 3 and 5 d post inoculation in CM plants. In contrast, induced *ETR4* mRNA accumulation in *jai1* was lower than in CM, whereas the

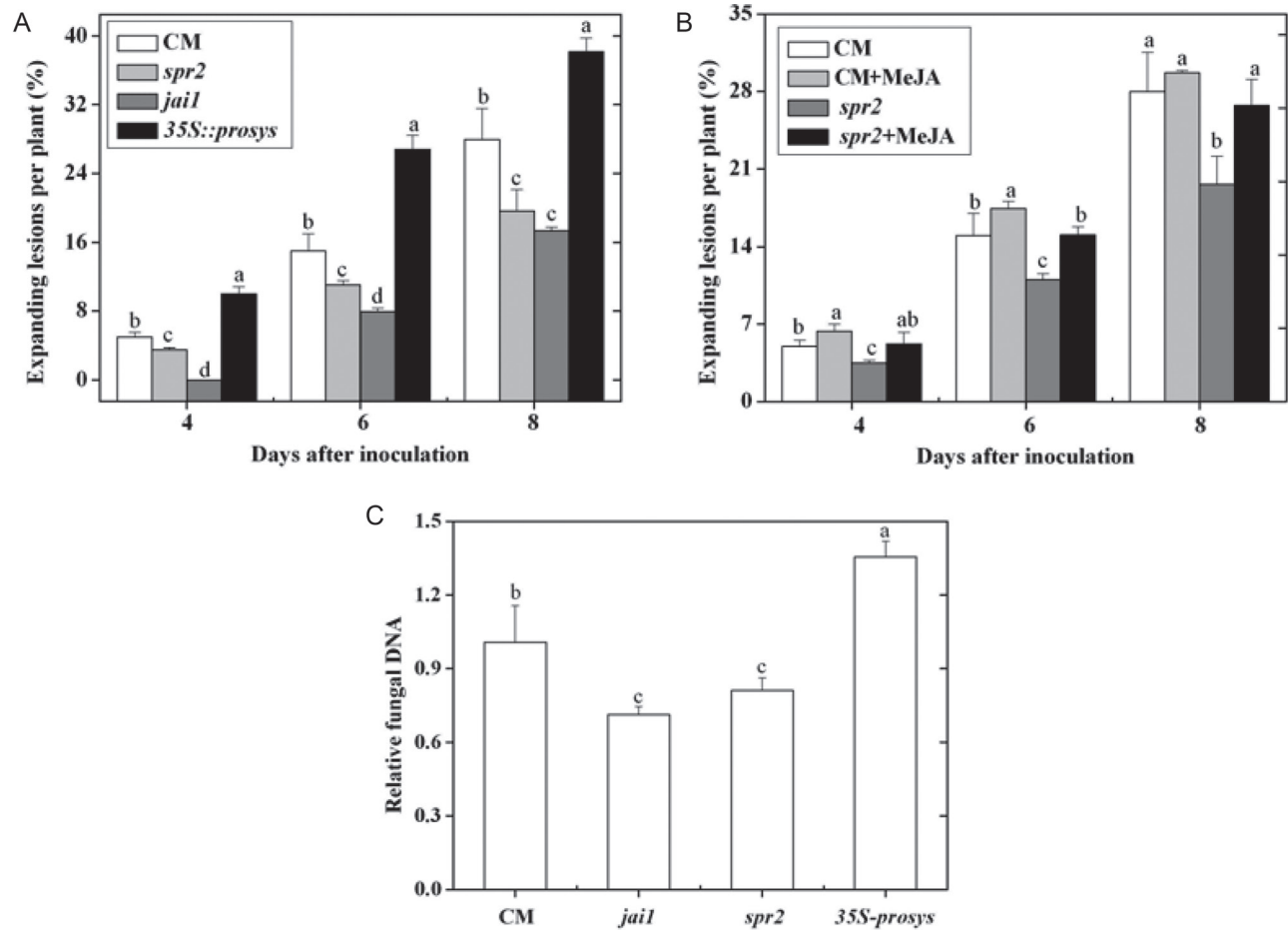


Fig. 2. The role of the JA pathway in tomato susceptibility to *A. alternata* f. sp. *lycopersici*. (A, B) Disease development in CM, *spr2*, *jai1*, and *35S::prosys* plants (A) and effect of application of MeJA to CM and *spr2* plants (B) at 4, 6, and 8 d post inoculation. (C) Fungal biomass in CM, *spr2*, *jai1*, and *35S::prosys* plants at 6 d post inoculation. The amount of fungal DNA was quantified by qPCR using *ALT1* gene primers. Error bars indicate standard deviation from the mean of three replicates. For each time point in A and B, letters indicate significant differences among treatments; in C, letters indicate significant differences among treatments ($P < 0.05$, Duncan's multiple range test).

expression of *NR* in *jai1* was not obviously altered in comparison with control plants at 0h. The expression of *ERFI* in CM was strongly induced and peaked at 1 d and the *ERFI* expression in *jai1* was greatly reduced at all the time points examined compared with CM (Fig. 4F). Together, these results indicate that both ET biosynthesis and the signalling pathway are strongly decreased in *jai1* leaves inoculated with AAL as compared to wide-type CM.

Enhanced susceptibility to AAL by JA is partly dependent on ET signal

To further test whether ET and JA act independently or cooperatively during AAL infection, *spr2* and *35S::prosys* plants were sprayed with ACC or silver thiosulphate before AAL inoculation, respectively. As shown in Fig. 5A, as with the similar trends for CM with ACC or silver thiosulphate pretreatment (Fig. 1A), *spr2* pretreated with ACC exhibited enhanced susceptibility at 4 and 6 d after inoculation, whereas application of silver thiosulphate enhanced the resistance of

35S::prosys to AAL, indicating that the enhanced susceptibility to this fungus by ET is not dependent on JA accumulation or response. Furthermore, there were increased expanding lesions and fungal biomass in ACC-pretreated *jai1* plants compared with untreated *jai1* (Fig. 5B, C), indicating that the role of ET in susceptibility to AAL is independent of the JAI1 receptor.

To obtain endogenous ET-deficient or ET-insensitive tomato plants, this study repetitively pretreated of CM plants from the cotyledon stage to the five-leaf stage, with ET biosynthesis inhibitor CoCl_2 or ET action inhibitor silver thiosulphate. The test showed that moderate concentrations of CoCl_2 at 0.1 mM and silver thiosulphate at 1 mM are most effective (Supplementary Fig. S2). Subsequently, the ET-deficient and ET-insensitive plants were pretreated with MeJA or H_2O alone to assess the interaction between the two signalling pathways. As shown in Fig. 5D, blocking ET perception or biosynthesis markedly reduced the expanding lesions, and pretreatment of ET-deficient or -insensitive plants with exogenous MeJA resulted in an increase of

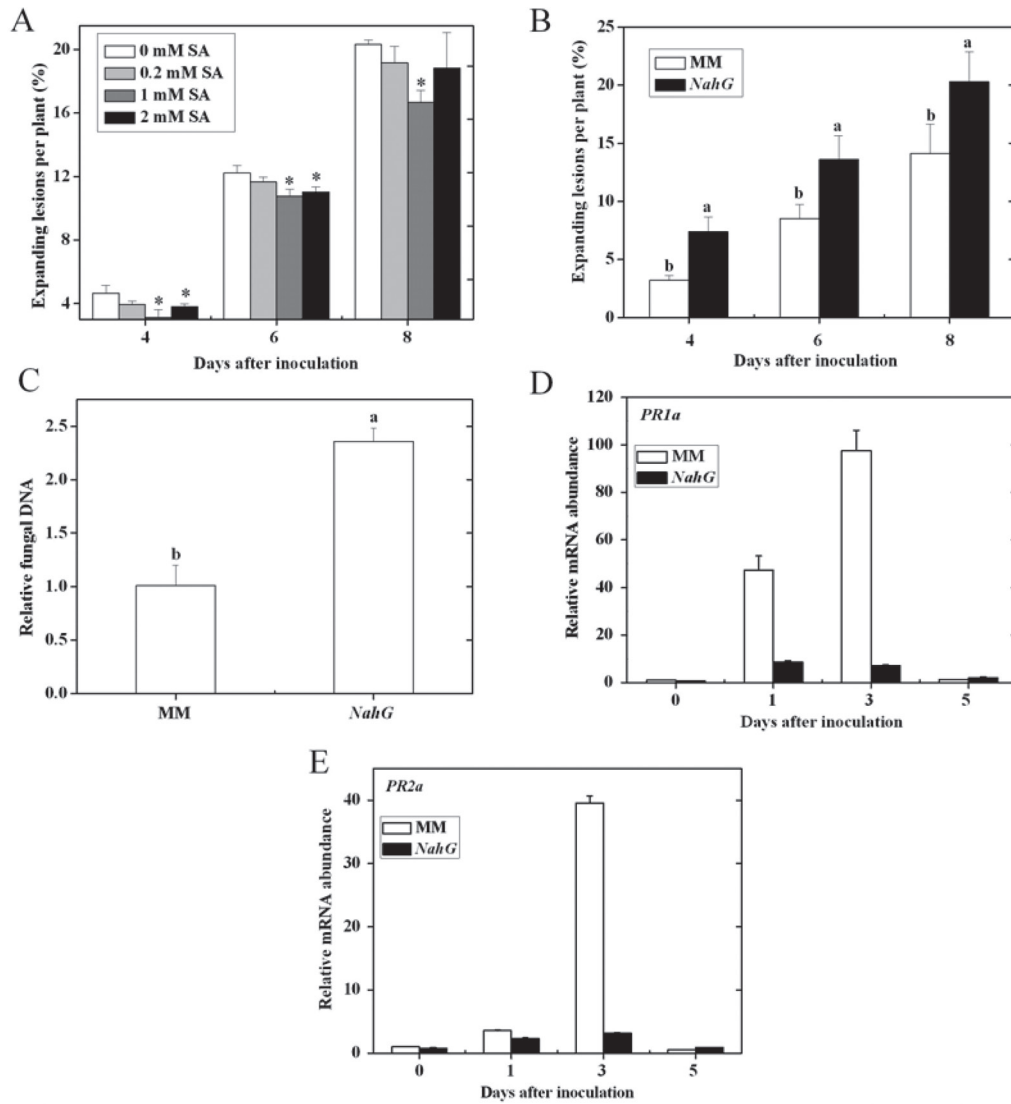


Fig. 3. The role of the SA pathway in tomato resistance to *A. alternata* f. sp. *lycopersici*. (A) Effects of various concentrations of SA application on the disease development in CM plants. (B) Disease development in the *NahG* transgenic line and MM plants. (C) Fungal biomass in MM and *NahG* transgenic plants at 6 d post inoculation. Error bars indicate standard deviation from the mean of three replicates. In A, asterisks indicate significant differences as compared with the water-treated control at the same time point ($P < 0.05$; Student's t-test), the experiment was repeated twice and similar results were obtained; for each time point in B, letters indicate significant differences among treatments ($P < 0.05$, Duncan's multiple range test); in C, letters indicate significant differences among treatments ($P < 0.05$, Duncan's multiple range test). (D, E) Transcription patterns of SA-regulated *PR* genes *PR1a* and *PR2a*, respectively, in MM and *NahG* plants at 0, 1, 3, and 5 d post inoculation.

necrotic lesion formation and restored disease symptoms to nearly the water-treated control level, suggesting that the role of JA response in tomato susceptibility is partly dependent on the ET signal and that the JA pathway also can promote tomato susceptibility to AAL alone.

SA suppresses ET signalling pathway during AAL infection

In *Arabidopsis*, SA and ET may function together to coordinate the induction of several defence-related genes (reviewed in Kunkel and Brooks, 2002). Here, since the role of ET signalling in tomato susceptibility to AAL were opposite

to SA, it was of interest to test whether the SA-enhanced resistance to this fungus was correlated with reduced ET signalling.

To determine the role of SA in ET-induced susceptibility to AAL, *NahG* transgenic plants were sprayed with silver thiosulphate before AAL inoculation. Silver thiosulphate application markedly decreased the expanding lesions in *NahG* (Fig. 6A). In addition, application of 1 mM SA did not significantly influence the disease symptoms in the ET-overproducing mutant *epi* (Fig. 6B), suggesting that the ET signal might be an effective factor in SA-induced defence response.

Furthermore, this study detected the expression of genes involved in ET biosynthesis or signalling in *NahG* and its WT

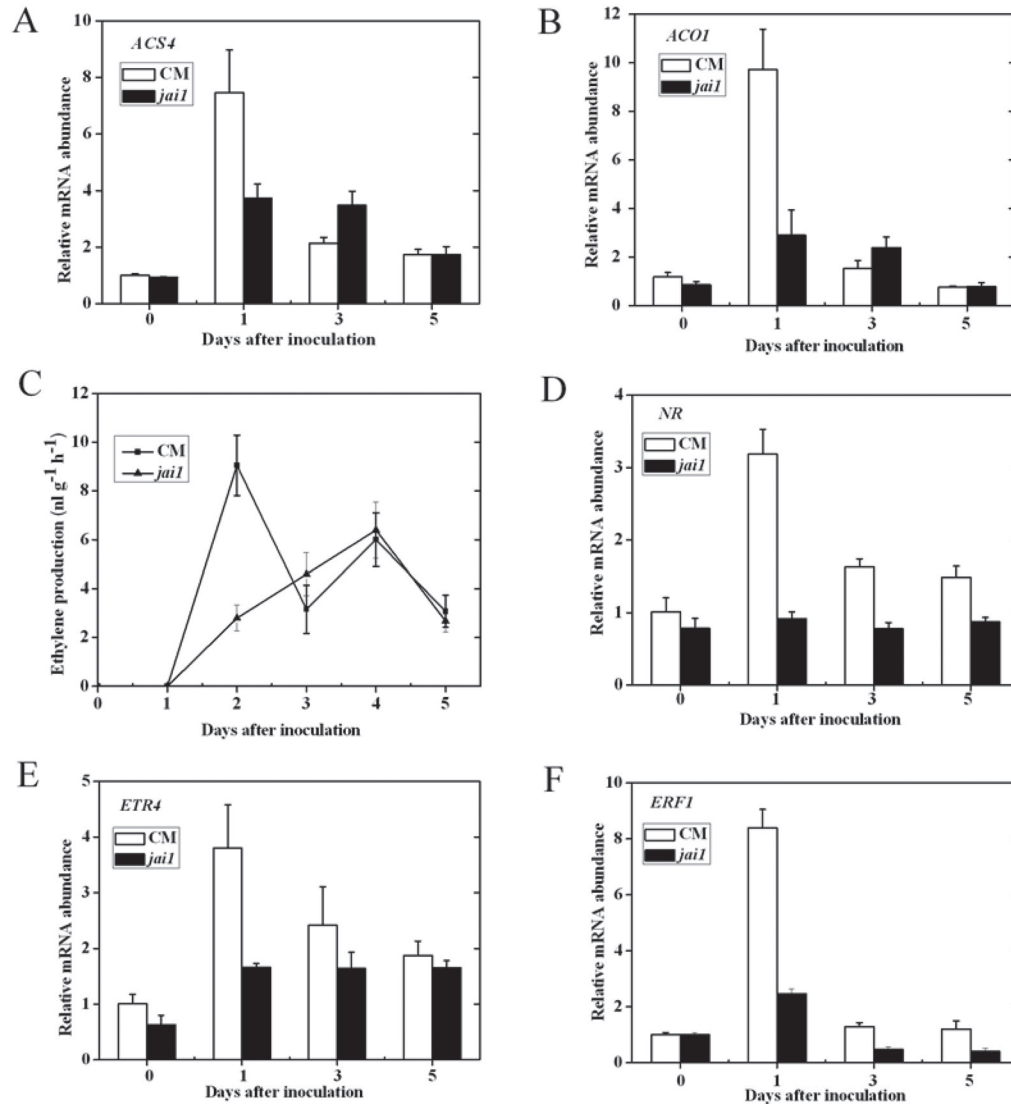


Fig. 4. Transcription patterns of ET-related genes and ET production in *jai1* and CM plants in response to *A. alternata* f. sp. *lycopersici*. Transcription patterns of *ACS4* (A) *ACO1* (B), *NR* (D), *ETR4* (E), and *ERF1* (F) in CM and *jai1* plants at 0, 1, 3, and 5 d post infection. (C) ET production in *jai1* and CM leaves during infection in a time-course experiment.

line cv. MM after AAL infection. As shown in Fig. 7A and B, the transcript levels of ET biosynthetic genes *ACS2* and *ACO1* were upregulated in *NahG* as compared with MM, clearly indicating that SA suppresses ET biosynthesis during AAL infection. Similarly to *ACS2* and *ACO1*, the expression abundance of the ET-responsive genes *NR*, *ETR4*, and *ERF1* was increased in *NahG* relative to MM (Fig. 7C–E). The above results suggest that enhanced resistance to AAL by SA might be associated with decreased ET response.

Discussion

Upon pathogen attack, infected plant cells generate signaling molecules to initiate defence mechanisms in surrounding cells to limit pathogen spread (Kunkel and Brooks, 2002; Koornneef and Pieterse, 2008). Three signalling molecules, SA, JA, and ET, play key roles in mediating disease resistance

(e.g. against necrotrophic fungal pathogens) (Asai et al., 2000). The disease of *Alternaria* stem canker is a problem primarily for coastal-grown tomatoes in California and some areas in China. There is limited information on the role of plant hormones in plant defence against this toxin-producing fungus. The present study investigated the roles of ET, JA, and SA as well as their interactions in modulating susceptibility of tomato plants to AAL.

The plant hormone ET is an important signal molecule in plant–pathogen interaction but the role of ET is largely controversial (Diaz et al. 2002). The *Arabidopsis ethylene insensitive2* (*ein2*) mutant exhibits increased susceptibility to *B. cinerea* and *E. carotovora*, but decreased symptoms when infected with *P. syringae* or *Xanthomonas campestris* pv. *campestris* (Kunkel and Brooks, 2002). Lund et al. (1998) reported that ET-insensitive tomato *Nr* shows reduced disease symptoms upon infection by the bacterial pathogens *P. syringae* pv. *tomato* and *Xanthomonas campestris* pv.

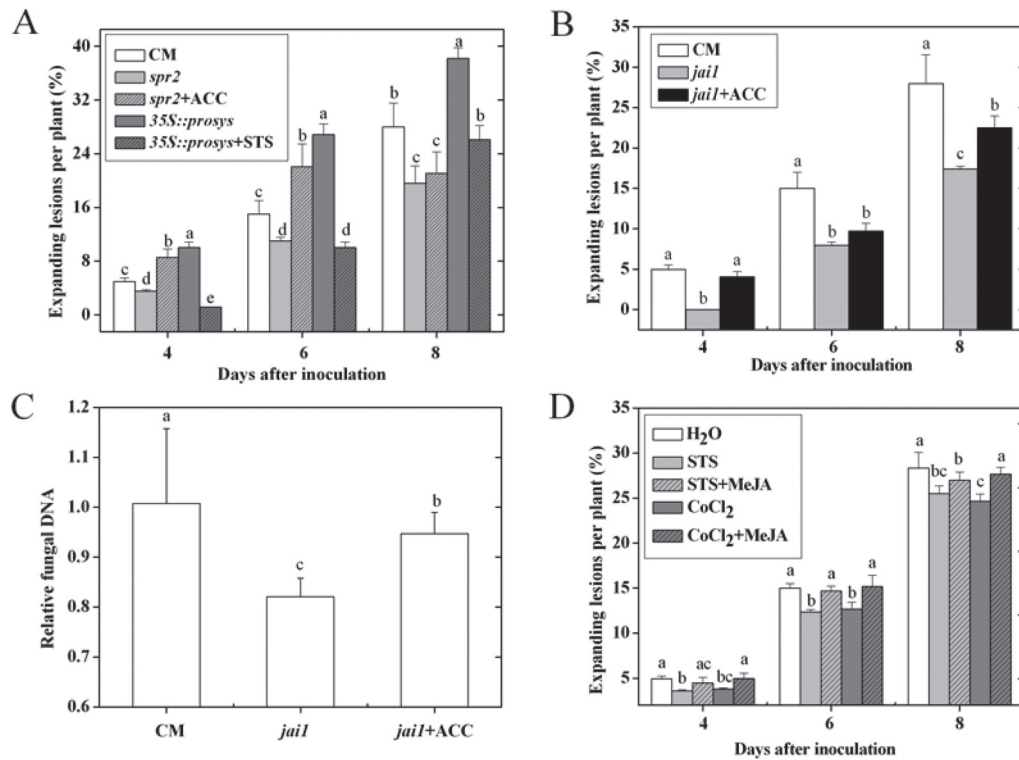


Fig. 5. Effects of ET response to JA mutants or *35S::prosys* and MeJA application to ET-biosynthetic or -insensitive tomato plants on susceptibility to *A. alternata* f. sp. *lycopersici*. (A) The effect of exogenous ET precursor ACC on *spr2* and ET action inhibitor silver thiosulphate on *35S::prosys* susceptibility to infection. (B) The effect of ACC on *jai1* susceptibility to infection. (C) Fungal biomass in CM, *jai1*, and ACC-pretreated *jai1* plants at 6 d post inoculation. (D) The effect of exogenous MeJA application to ET-biosynthetic or -insensitive tomato plants on disease development. Error bars indicate standard deviation from the mean of three replicates. For each time point in A, B, and D, letters indicate significant differences among treatments; in C, letters indicate significant differences among treatments ($P < 0.05$, Duncan's multiple range test). The experiment was repeated once and similar results were obtained.

vesicatoria. The studies using *Arabidopsis* and tomato ET mutants demonstrated that both ET perception and signaling are required for resistance against or susceptibility to pathogens, depending on the plant-pathogen interaction (Lund *et al.*, 1998; Thomma *et al.*, 1999; Diaz *et al.*, 2002). In the present study, the results suggest a novel role for ET in

modulating susceptibility of tomato plants to AAL (Fig. 1). ET action can be regulated by changes of its biosynthesis and perception (Klee, 2002). The present study also observed that the production of ET is one of the earliest plant responses (Fig. 4C). Combined with the previous reports (Moore *et al.*, 1999; Zhang *et al.*, 2011), it seemed that production of ET

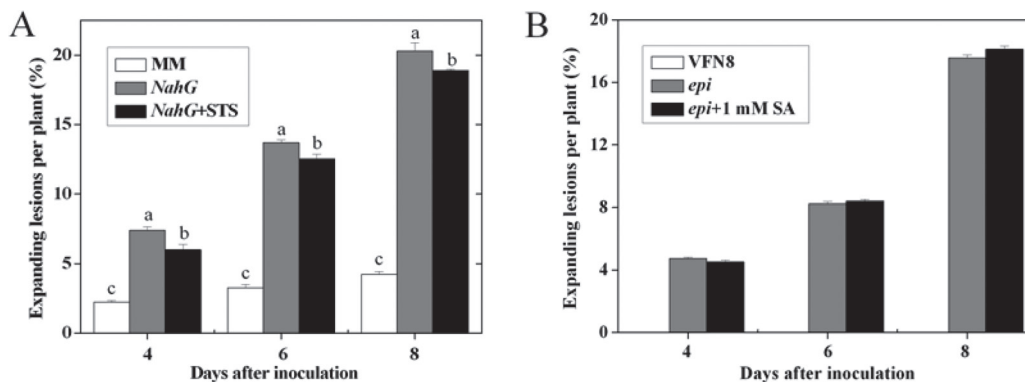


Fig. 6. Effects of ET action inhibitor silver thiosulphate application to *NahG* transgenic line (A) and SA treatment of *epi* mutant (B) on disease development in tomato plants. Error bars indicate standard deviation from the mean of three replicates. In A, letters indicate significant differences among treatments ($P < 0.05$, Duncan's multiple-range test). The experiment was repeated once and similar results were obtained. For each experiment, data were analysed separately. Results of one representative experiment are shown.

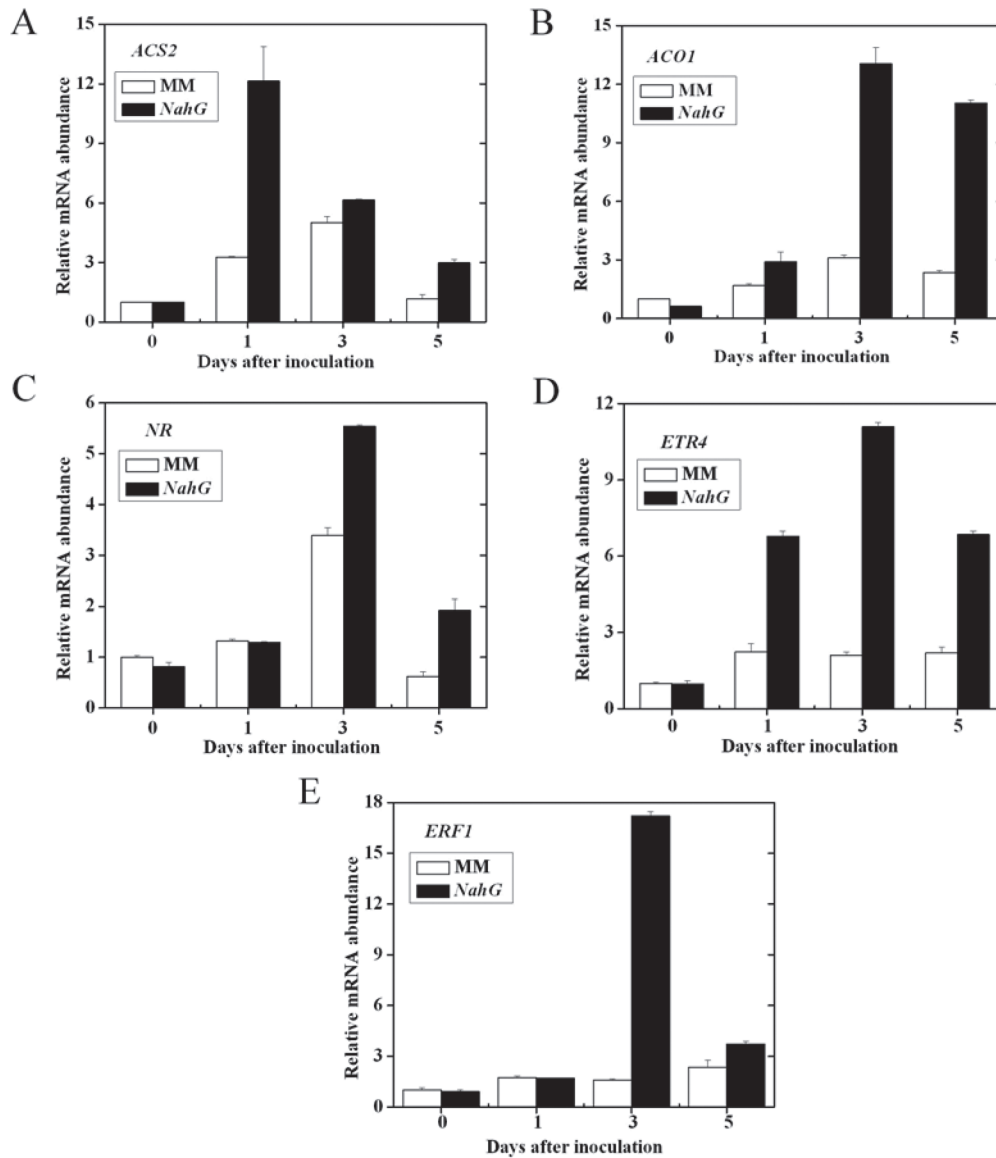


Fig. 7. Transcription patterns of ET-biosynthetic genes *ACS2* and *ACO1* and ET-regulated genes *NR*, *ETR4*, and *ERF1* in *NahG* and MM plants in response to *A. alternata* f. sp. *lycopersici*. Transcript accumulation of *ACS2* (A), *ACO1* (B), *NR* (C), *ETR4* (D), and *ERF1* (E) in *A. alternata* f. sp. *lycopersici*-infected MM and *NahG* plants at 0, 1, 3, and 5 d post infection.

could be induced by the pathogen or the AAL toxin at the earliest stage, which promoted AAL-toxin-induced cell death. Then, the necrotrophic pathogen AAL extracted nutrients from the dead host cells and produced more AAL toxin, which in turn enhanced cell death.

Necrotrophic fungal pathogens infect and kill host tissue and extract nutrients from the dead host cells, while biotrophic fungal pathogens colonize living plant tissue and obtain nutrients from living host cells. Classically, SA signalling triggers resistance against biotrophic and hemibiotrophic pathogens, whereas a combination of JA and ET signalling activates resistance against necrotrophic pathogens (Glazebrook, 2005). However, from the present data, it can be concluded that the JA signalling pathway is involved in tomato susceptibility to AAL (Fig. 2). In a recent report by Egusa *et al.* (2009), the detached tomato leaflets of JA-deficient mutant

def1 show increased resistance to AAL, which is consistent with the present results. Additionally, it is shown here that SA mediates resistance of tomato plants to AAL (Fig. 3A–C). Egusa *et al.* (2009) reported that the exogenous addition of SA to spore suspensions does not affect susceptibility of detached tomato leaves to AAL, which is contradictory to the present results, and it should be noted that their methods of fungal inoculation and SA application were different from this study's. Furthermore, exogenous 0.2 mM SA application significantly inhibited AAL-toxin-induced cell death in detached leaflets (Supplementary Fig. S3), suggesting that the enhanced resistance to AAL by SA might be associated with the inhibition of cell death. In several previously reported studies, elevated SA often enhances susceptibility to necrotrophic pathogens but promotes resistance to hemibiotrophs (Veronese *et al.*, 2004, 2006; El Oirdi *et al.*, 2011).

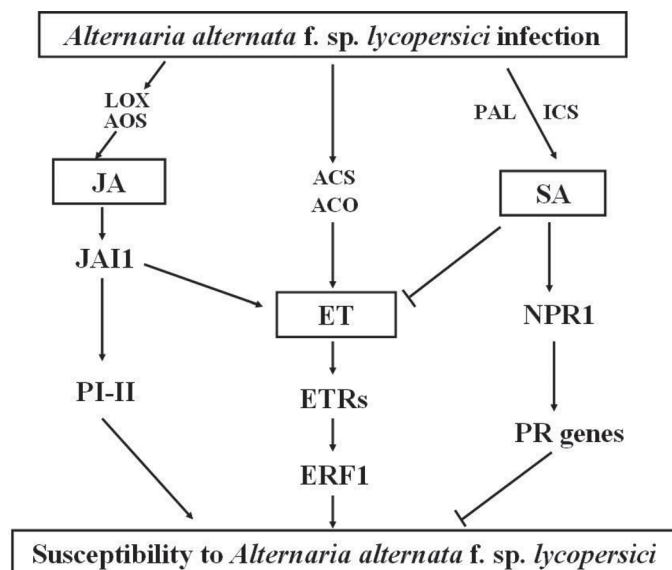


Fig. 8. A proposed model for the JA, SA, and ET defence signalling pathways and their interactions during *A. alternata* f. sp. *lycopersici* infection. Both ET and JAI1 receptor-dependent JA pathways are necessary for susceptibility, while SA signalling promotes resistance of tomato plants to infection. Furthermore, the JA and ET pathways act synergistically to promote the susceptibility, while SA promotes tomato resistance to AAL and antagonizes ET signalling during AAL infection. JA and SA affect tomato susceptibility to the pathogen partly through the action of ET. ET-mediated signalling plays a central role in determining the susceptibility of tomato to *A. alternata* f. sp. *lycopersici*.

In the present study, the roles of JA and SA in susceptibility of tomato plants to AAL were different from other plant and necrotrophic pathogen interactions (Thomma *et al.*, 1998; Diaz *et al.*, 2002; El Oirdi *et al.*, 2011). There could be two possible reasons for the discrepancies. First, most of the studies on the roles of JA and SA in plant resistance to necrotrophic pathogens have been carried out in the model plant *Arabidopsis* and there could be significance differences between *Arabidopsis* and tomato in their signalling pathways or defence mechanisms against necrotrophic pathogens. Second, most of the reported studies on the roles of JA and SA in plant responses to necrotrophic pathogens have used broad host-range necrotrophs such as *B. cinerea*. AAL, on the other hand, is a host-specific necrotroph, which produces a group of mycotoxins as a host-specific pathogenicity factor (Brandwagt *et al.*, 2002). The host-specific nature of the toxins and the pathogens may imply specific plant–pathogen interactions that require manoeuvring host physiology and viability at some stages of the infection cycle, a feature also found with hemibiotrophic pathogens.

The JA, SA, and ET pathways are involved in a complex signalling network in which the different pathways influence each other through positive or negative regulatory interactions. It allows plants to minimize energy costs and create a flexible signalling network for the defence responses to

different attackers (Kunkel and Brooks, 2002; Koornneef and Pieterse, 2008). Several studies provide evidence for positive interactions between JA and ET signalling (Kunkel and Brooks, 2002). For example, ET is required together with JA for defence against necrotrophic pathogens and for systemic resistance induced by root-colonizing bacteria, and they stimulate each other's biosynthesis in wound responses (reviewed in Tuominen *et al.*, 2004). Transcription factor ERF1 acts as an important signalling node integrating signals from the JA and ET pathways (Koornneef and Pieterse, 2008). Berrocal-Lobo and Molina (2004) demonstrated that *ERF1* is induced upon infection with *Fusarium oxysporum* and depends on both functionally intact ET and JA pathways. Similar results were observed in this study, in which the resistance of *jail1* was enhanced and ET response was reduced in *jail1* as compared to CM, suggesting that the ET and JA pathways probably cooperatively regulate tomato susceptibility to AAL. ACC application potentiated disease symptom development in the *spr2* mutants and silver thiosulphate alleviated the development of disease symptoms in the *35S::prosys* plants (Fig. 5A). Furthermore, exogenous MeJA application to ET-insensitive plants (obtained by repetitive pretreatment of CM plants with silver thiosulphate) and to ET-deficient plants (obtained by repetitive pretreatment of CM plants with CoCl_2) before AAL inoculation both resulted in similar percentages of expanding lesions to the untreated control (Fig. 5D), but less than MeJA treatment alone (Fig. 2B), indicating that the effect of JA on susceptibility to AAL is not dependent on ET signalling completely.

Although the induction of SA-dependent *PR* gene expression does not require an intact ET signalling in *Arabidopsis*, ACC application enhanced the level of SA-induced *PR1* expression in a dose-dependent manner (De Vos *et al.*, 2006). Moreover, ET was shown to be essential for the onset of SA-dependent systemic acquired resistance against tobacco mosaic virus in tobacco (Verberne *et al.*, 2003). The accumulation of SA in *X. campestris* pv. *vesicatoria*-infected tomato plants is dependent on ET synthesis (O'Donnell *et al.*, 2003). These results suggest that ET positively regulates the SA-induced defence response. However, the ET signalling also negatively affects SA-dependent responses: the basal level of *PR-1* mRNA appears to be significantly elevated in *Arabidopsis ein2* plants (reviewed in Kunkel and Brooks, 2002). Here in the interaction between tomato and AAL, they seem to be antagonistic. First, compared with MM, *NahG* transgenic plants exhibited higher expression of ET-biosynthetic and -responsive genes (Fig. 7), indicating that SA-enhanced resistance to AAL might be correlated with a decrease in the ET signalling pathway. Secondly, pretreatment of *NahG* plants with silver thiosulphate significantly alleviated the development of disease symptoms (Fig. 6A), while exogenous application of SA to the *epi* mutant had no significant effect on disease development (Fig. 6B), indicating that ET might play an important role in SA-induced defence response during AAL infection.

There is evidence for both positive and negative interactions between the SA and JA pathways. JA antagonizes SA-dependent pathogen defences in the course of *P. syringae*

infection in tomato (Kunkel and Brooks, 2002). In *Arabidopsis*, the reduced susceptibility of *jml1*, a JA-insensitive mutant to *P. syringae*, is caused by hyperactivation of *PR* and elevated SA accumulation (Laurie-Berry *et al.*, 2006). In this study, to test the interactions between the SA and JA pathways, the expression of SA-biosynthetic or -regulated genes were detected in *jail* and CM following AAL infection. The results showed that there were similar expression patterns of *PAL* (encoding phenylalanine ammonia-lyase, Asai *et al.*, 2000) or *ICS* (isochorismate synthase, Uppalapati *et al.*, 2007) in *jail* and CM, and no obvious differences were observed between them following AAL infection (Supplementary Fig. S4A, B). Moreover, the expression of the SA-regulated genes *NPRI* and *PR1a* was down-regulated in *jail* compared with CM (Supplementary Fig. S4C, D), suggesting that SA-mediated defence signalling is reduced in *jail* relative to CM during AAL infection. Collectively, these results suggest that enhanced resistance against AAL in *jail* plants is independent of activated SA-mediated defence response. A similar result was observed in *Arabidopsis*, in which COI1 receptor-mediated *F. oxysporum* resistance was not attributed to SA-dependent defence (Thatcher *et al.*, 2009).

In conclusion, a hypothetical model to summarize the roles of the ET, JA, and SA pathways as well as the signalling crosstalk during the tomato–AAL interaction is proposed (Fig. 8). Challenge of tomato plants by fungal AAL starts a cascade of signalling events that involve the synthesis and subsequent activation of the ET, JA, and SA pathways. The ET response and JAI1-dependent JA signalling enhance the susceptibility of tomato plants to AAL, while SA pathway is involved in resistance against AAL. Furthermore, the JA and ET pathways act synergistically to promote tomato susceptibility, while SA promotes tomato resistance to AAL and antagonizes ET signalling during AAL infection. JA and SA affect the susceptibility of tomato to the pathogen partly through the action of ET. ET-mediated signalling might be more effective in determining tomato susceptibility to AAL.

Supplementary material

Supplementary data are available at *JXB* online.

Supplementary Fig. S1. Transcription patterns of the JA-biosynthetic genes *AOS2* and *LOXD* and the JA-regulated gene *PI-II* in the leaves of *jail* and CM post AAL infection.

Supplementary Fig. S2. Effects of various concentrations of CoCl_2 and silver thiosulphate application on disease development in CM plants.

Supplementary Fig. S3. Symptom of CM leaflets after 0.2 μM AAL toxin application or co-treatment with AAL toxin and 0.2 mM SA.

Supplementary Fig. S4. Transcription patterns of SA-biosynthetic genes *PAL* and *ICS* and JA-regulated genes *NPRI* and *PR1a* in the leaves of *jail* and CM post AAL infection.

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References

- Asai T, Stone JM, Heard JE, Kovtun Y, Yorgey P, Sheen J, Ausubel FM. 2000. Fumonisin B1-induced cell death in *Arabidopsis* protoplasts requires jasmonate-, ethylene-, and salicylate-dependent signaling pathways. *The Plant Cell* **12**, 1823–1835.
- Bellés JM, Pérez-Amador MA, Carbonell J, Conejero V. 1993. Correlation between ornithine decarboxylase and putrescine in tomato plants infected by citrus exocortis viroid or treated with ethephon. *Plant Physiology* **102**, 933–937.
- Berrocal-Lobo M, Molina A. 2004. Ethylene response factor 1 mediates *Arabidopsis* resistance to the soilborne fungus *Fusarium oxysporum*. *Molecular Plant–Microbe Interactions* **17**, 763–770.
- Brading PA, Hammond-Kosack KE, Parr A, Jones JD. 2000. Salicylic acid is not required for Cf-2- and Cf-9-dependent resistance of tomato to *Cladosporium fulvum*. *The Plant Journal* **23**, 305–318.
- Brandwagt BF, Kneppers TJA, Nijkamp HJJ, Hille J. 2002. Overexpression of the tomato *Asc-1* gene mediates high insensitivity to AAL toxins and fumonisin B1 in tomato hairy roots and confers resistance to *Alternaria alternata* f. sp. *lycopersici* in *Nicotiana umbratica* plants. *Molecular Plant–Microbe Interactions* **15**, 35–42.
- De Vos M, Van Zaanen W, Koornneef A, Korzelijs JP, Dicke M, Van Loon LC, Pieterse CMJ. 2006. Herbivore-induced resistance against microbial pathogens in *Arabidopsis*. *Plant Physiology* **142**, 352–363.
- Diaz J, ten Have A, van Kan JAL. 2002. The role of ethylene and wound signaling in resistance of tomato to *Botrytis cinerea*. *Plant Physiology* **129**, 1341–1351.
- Doares SH, Navarraz-Vasquez J, Conconi A, Ryan CA. 1995. Salicylic acid inhibits synthesis of proteinase inhibitors in tomato leaves induced by systemin and jasmonic acid. *Plant Physiology* **108**, 1741–1746.
- Egusa M, Ozawa R, Takabayashi J, Otani H, Kodama M. 2009. The jasmonate signaling pathway in tomato regulates susceptibility to a toxin-dependent necrotrophic pathogen. *Planta* **229**, 965–976.
- El Oirdi M, El Rahman TA, Rigano L, El Hadrami A, Rodriguez MC, Daayf F, Vojnov A, Bouarab K. 2011. *Botrytis cinerea* manipulates the antagonistic effects between immune pathways to promote disease development in tomato. *The Plant Cell* **23**, 2405–2421.
- Fujino DW, Burger DW, Yang SF, Bradford KJ. 1988. Characterization of an ethylene overproducing mutant of tomato (*Lycopersicon esculentum* Mill. cultivar VFN8). *Plant Physiology* **88**, 774–779.

- Glazebrook J.** 2005. Contrasting mechanisms of defense against biotrophic and necrotrophic pathogens. *Annual Review of Phytopathology* **43**, 205–227.
- Howe GA, Ryan CA.** 1999. Suppressors of systemin signaling identify genes in the tomato wound response pathway. *Genetics* **153**, 1411–1421.
- Klee HJ.** 2002. Control of ethylene-mediated processes in tomato at the level of receptors. *Journal of Experimental Botany* **53**, 2057–2063.
- Kloek AP, Verbsky ML, Sharma SB, Schoelz JE, Vogel J, Klessig DF, Kunkel BN.** 2001. Resistance to *Pseudomonas syringae* conferred by an *Arabidopsis thaliana coronatine-insensitive (coi1)* mutation occurs through two distinct mechanisms. *The Plant Journal* **26**, 509–522.
- Koornneef A, Pieterse CMJ.** 2008. Cross talk in defense signaling. *Plant Physiology* **146**, 839–844.
- Kunkel BN, Brooks DM.** 2002. Cross talk between signaling pathways in pathogen defense. *Current Opinion in Plant Biology* **5**, 325–331.
- Laurie-Berry N, Joardar V, Street IH, Kunkel BN.** 2006. The *Arabidopsis thaliana jasmonate insensitive 1* gene is required for suppression of salicylic acid-dependent defenses during infection by *Pseudomonas syringae*. *Molecular Plant–Microbe Interactions* **19**, 789–800.
- Li CY, Liu G, Xu C, Lee GI, Bauer P, Ling HQ, Ganai MW, Howe GA.** 2003. The tomato suppressor of prosystemin-mediated responses2 gene encodes a fatty acid desaturase required for the biosynthesis of jasmonic acid and the production of a systemic wound signal for defense gene expression. *The Plant Cell* **15**, 1646–1661.
- Li CY, Williams MM, Loh YT, Lee GI, Howe GA.** 2002. Resistance of cultivated tomato to cell content-feeding herbivores is regulated by the octadecanoid-signaling pathway. *Plant Physiology* **130**, 494–503.
- Li L, Zhao Y, McCaig BC, Wingerd BA, Wang J, Whalon ME, Pichersky E, Howe GA.** 2004. The tomato homolog of CORONATINE-INSENSITIVE1 is required for the maternal control of seed maturation, jasmonate-signaled defense responses, and glandular trichome development. *The Plant Cell* **16**, 126–143.
- Lorenzo O, Piqueras R, Sanchez-Serrano JJ, Solano R.** 2003. ETHYLENE RESPONSE FACTOR1 integrates signals from ethylene and jasmonate pathways in plant defense. *The Plant Cell* **15**, 165–178.
- Lund ST, Stall RE, Klee HJ.** 1998. Ethylene regulates the susceptible response to pathogen infection in tomato. *The Plant Cell* **10**, 371–382.
- McDowell JM, Dangl JL.** 2000. Signal transduction in the plant immune response. *Trends in Biochemical Sciences* **25**, 79–82.
- Moore T, Martineau B, Bostock RM, Lincoln JE, Gilchrist DG.** 1999. Molecular and genetic characterization of ethylene involvement in mycotoxin-induced plant cell death. *Physiological and Molecular Plant Pathology* **54**, 73–85.
- Mur LAJ, Kenton P, Atzorn R, Miersch O, Wasternack C.** 2006. The outcomes of concentration-specific interactions between salicylate and jasmonate signaling include synergy, antagonism, and oxidative stress leading to cell death. *Plant Physiology* **140**, 249–262.
- Niki T, Mitsuhashi I, Seo S, Ohtsuba N, Ohashi Y.** 1998. Antagonistic effect of salicylic acid and jasmonic acid on the expression of pathogenesis-related (PR) protein genes in wounded mature tobacco leaves. *Plant Cell Physiology* **39**, 500–507.
- Nürnberg T, Lipka V.** 2005. Non-host resistance in plants: new insights into an old phenomenon. *Molecular Plant Pathology* **6**, 335–345.
- O'Donnell PJ, Schmelz E, Block A, Miersch O, Wasternack C, Jones JB, Klee HJ.** 2003. Multiple hormones act sequentially to mediate a susceptible tomato pathogen defense response. *Plant Physiology* **133**, 1181–1189.
- Pearce G, Strydom D, Johnson S, Ryan CA.** 1991. A polypeptide from tomato leaves induces wound-inducible proteinase inhibitor proteins. *Science* **253**, 895–897.
- Schenk PM, Kazan K, Wilson I, Anderson JP, Richmond T, Somerville SC, Manners JM.** 2000. Coordinated plant defense responses in *Arabidopsis* revealed by microarray analysis. *Proceedings of the National Academy of Sciences, USA* **97**, 11655–11660.
- Sheard LB, Tan X, Mao H, et al.** 2010. Jasmonate perception by inositol phosphate-potentiated COI1-JAZ co-receptor. *Nature* **468**, 400–405.
- Spoel SH, Johnson JS, Dong X.** 2007. Regulation of tradeoffs between plant defense against pathogens with different lifestyles. *Proceedings of the National Academy of Sciences, USA* **104**, 18842–18847.
- Sun JQ, Jiang HL, Li CY.** 2011. Systemin/jasmonate-mediated systemic defense signaling in tomato. *Molecular Plant* **4**, 607–615.
- Thatcher LF, Manners JM, Kazan K.** 2009. *Fusarium oxysporum* hijacks COI1-mediated jasmonate signaling to promote disease development in *Arabidopsis*. *The Plant Journal* **58**, 927–939.
- Thomma BPHJ, Eggermont K, Penninckx IA, Mauch-Mani B, Vogelsang R, Cammue BPA, Broekaert WF.** 1998. Separate jasmonate-dependent and salicylate-dependent defense-response pathways in *Arabidopsis* are essential for resistance to distinct microbial pathogens. *Proceedings of the National Academy of Sciences, USA* **95**, 15107–15111.
- Thomma BPHJ, Eggermont K, Tierens KFMJ, Broekaert WF.** 1999. Requirement of functional *ethylene-insensitive 2* gene for efficient resistance of *Arabidopsis* to infection by *Botrytis cinerea*. *Plant Physiology* **121**, 1093–1101.
- Tuominen H, Overmyer K, Keinänen M, Kollist H, Kangasjärvi J.** 2004. Mutual antagonism of ethylene and jasmonic acid regulates ozone-induced spreading cell death in *Arabidopsis*. *The Plant Journal* **39**, 59–69.
- Uppalapati SR, Ishiga Y, Wangdi T, Kunkel BN, Anand A, Mysore KS, Bender CL.** 2007. The phytotoxin coronatine contributes to pathogen fitness and is required for suppression of salicylic acid accumulation in tomato inoculated with *Pseudomonas syringae* pv. *tomato* DC3000. *Molecular Plant–Microbe Interactions* **20**, 955–965.
- Verberne MC, Hoekstra J, Bol JF, Linthorst HJM.** 2003. Signaling of systemic acquired resistance in tobacco depends on ethylene perception. *The Plant Journal* **35**, 27–32.

- Veronese P, Chen X, Bluhm B, Salmeron J, Dietrich R, Mengiste T.** 2004. The BOS loci of *Arabidopsis* are required for resistance to *Botrytis cinerea* infection. *The Plant Journal* **40**, 558–574.
- Veronese P, Nakagami H, Bluhm B, AbuQamar S, Chen X, Salmeron J, Dietrich RA, Hirt H, Mengiste T.** 2006. The membrane-anchored BOTRYTIS-INDUCED KINASE1 plays distinct roles in *Arabidopsis* resistance to necrotrophic and biotrophic pathogens. *The Plant Cell* **18**, 257–273.
- van Wees SC, Glazebrook J.** 2003. Loss of non-host resistance of *Arabidopsis NahG* to *Pseudomonas syringae* pv. *phaseolicola* is due to degradation products of salicylic acid. *The Plant Journal* **33**, 733–742.
- Wilkinson JQ, Lanahan MB, Yen HC, Giovannoni JJ, Klee HJ.** 1995. An ethylene-inducible component of signal transduction encoded by *Never-ripe*. *Science* **270**, 1807–1809.
- Yang SF, Hoffman NE.** 1984. Ethylene biosynthesis and its regulation in higher plants. *Annual Review of Plant Physiology* **35**, 155–189.
- Yu D, Chen C, Chen Z.** 2001. Evidence for an important role of WRKY DNA binding proteins in the regulation of *NPR1* gene expression. *The Plant Cell* **13**, 1527–1539.
- Zhang LP, Jia CG, Liu LH, Zhang ZM, Li CY, Wang QM.** 2011. The involvement of jasmonates and ethylene in *Alternaria alternata* f. sp. *lycopersici* toxin-induced tomato cell death. *Journal of Experimental Botany* **62**, 5405–5418.