

Induction of tomato *Jasmonate-Resistant 1-Like 1* gene expression can delay the colonization of *Ralstonia solanacearum* in transgenic tomato

Jhy-Gong WANG and Hsu-Liang HSIEH*

Institute of Plant Biology, College of Life Science, National Taiwan University, Taipei 106, Taiwan

(Received April 8, 2011; Accepted October 6, 2011)

ABSTRACT. The bacterial wilt in tomato caused by *Ralstonia solanacearum* infection is common and widespread, especially in hot and humid environments. Combating this disease is difficult due to unstable host resistance and the variation and diversity of the bacterial strains. Thus, the molecular mechanisms underlying tomato resistance against *Ralstonia solanacearum* remain unknown. Here, we isolated a homolog of tomato *Solanum lycopersicum* *Jasmonate-Resistant 1* (*SIJAR1*), named *SIJAR1-like 1* (*SIJRL1*), and generated transgenic tomato lines harboring an inducible promoter-driven *SIJRL1* construct. *SIJRL1* shares 99% amino acid identity with *SIJAR1*. Intriguingly, *SIJRL1* showed preferential expression in aerial parts and *SIJAR1* in roots. DNA gel blot analysis revealed multiple copies of *SIJRL1* in the tomato genome. Transgenic tomato containing a single copy of the transgene *SIJRL1* exhibited high levels of *SIJRL1* expression two days after dexamethasone (DEX) induction. Moreover, DEX-induced *SIJRL1* expression could delay the symptoms of tomato bacterial wilt, and efficiently reduce the amount of *Ralstonia* in stems. The phytohormone jasmonic acid may play a role in this resistance response. This study of inducible *SIJRL1* expression in transgenic tomato contributes to the molecular understanding of tomato resistance against bacterial wilt.

Keywords: Jasmonic acid; *Ralstonia solanacearum*; *SIJAR1*; Tomato bacterial wilt-*SIJAR1*.

INTRODUCTION

Ralstonia solanacearum, a soil-borne bacterium and one of the most devastating pathogens, causes a lethal disease known as bacterial wilt in more than 200 plant species in tropical, subtropical and temperate regions of the world. Its hosts are ornamentals, weeds, and crops, including tomato, potato, banana and pepper (Hayward, 1991; Schell, 2000; Deslandes et al., 2002). At the early stage of infection, *R. solanacearum* attaches to and enters the lateral roots where wounding has taken place. Subsequently, the bacterium invades the root cortex, translocates to the xylem vessels, and spreads rapidly throughout the vascular system of infected hosts. The bacterium then colonizes the xylem tissues of plants and causes wilt symptoms and death (Schell, 2000; Genin and Boucher, 2004). *R. solanacearum* is later released from the roots or the collapsed stems into the soil for the infection of new hosts.

Because of the serious impact *R. solanacearum* infection has on plants, several approaches have been used for control, including crop rotation (Adhikari and Basnyat, 1998), biocontrol agent application (Guo et al., 2004; Xue

et al., 2009), and resistant cultivar breeding via traditional or genetic engineering strategies (Chan et al., 2005; Thonquyet et al., 1996; Wang et al., 2000). Crop rotation has a limited effect on controlling *R. solanacearum* due to the its wide range of hosts. Although biological control using bacteria has drawn much attention, its efficiency is limited because of environmental factors such as temperature, moisture, and the storage of bacteria (Guo et al., 2004). Although traditional breeding systems have produced some resistant breeds, their numbers are still limited and some undesirable traits have occurred. Genetic engineering has been used to generate plants with high resistance against *R. solanacearum* infection. Chan et al. (2005) reported that transgenic tomatoes harboring an *Arabidopsis* thionine gene *THI2.1* driven by a fruit-inactive promoter displayed high resistance to this wilt bacteria.

In addition, previous studies suggested that plant hormones, including salicylic acid (SA) and ethylene (ET), play roles in wilt bacteria resistance. SA participates in *R. solanacearum* resistance in *Arabidopsis* in a gene-for-gene strategy (Deslandes et al., 2002; Deslandes et al., 2003) involving the bacterial PopP2 protein and *Arabidopsis* RRS1-R protein. The wilt bacteria first invade plant cells and release PopP2, a type III effector acting as an avirulence (Avr) protein, into the cytoplasm of plant cells. PopP2 then interacts with RRS1-R protein, a resistant

*Corresponding author: E-mail: hlhsieh@ntu.edu.tw; Tel: +886-2-33662540; Fax: +886-2-23918940.

(R) protein containing a WRKY domain, to form a complex. The complex enters the nucleus to activate defense-related genes, thus leading to enhanced resistance against *R. solanacearum* (Deslandes et al., 2002; Deslandes et al., 2003). In the ET signaling pathway, Ethylene-Insensitive 2 (EIN2) is a positive regulator and degraded via 26S proteasome (Qiao et al., 2009; Stepanova and Alonso, 2009). Previous reports have shown the *ein2-1* mutant with delayed wilt symptoms after inoculation with *R. solanacearum* (Hirsch et al., 2002). Furthermore, some effectors of wilt bacteria can promote hormone production and influence plant defense responses (Hirsch et al., 2002). For example, inoculation with wilt bacteria induced the production of ET, leading to the induced expression of *Ethylene Response Factor 1* (*ERF1*) and *Pathogen Response Gene 4* (Valls et al., 2006). Therefore, phytohormone biosynthesis and signaling play important roles in defending against *R. solanacearum* infection.

In addition to SA and ET, another biotic-stressed hormone, jasmonic acid (JA), plays an important role in the control of biotic invaders, such as necrotrophic pathogens and herbivores (as reviewed in Katsir et al., 2008). Previous studies have reported that pathogen infection may induce the production of JA-isoleucine (JA-Ile), an active form of JA, in *Arabidopsis*, thus leading to increased expression of JA-responsive genes. Moreover, low levels of JA-Ile in the *jar1-1* mutant suppressed JA responses and reduced the resistance against microbial pathogens and herbivores (Staswick and Tiriyaki, 2004; Browse, 2009). JA-Ile formation is mediated by jasmonate-resistant 1 (*JAR1*) protein, which conjugates JA and isoleucine (Staswick et al., 2002; Staswick and Tiriyaki, 2004). The binding of JA-Ile and coronatine-insensitive 1 (*COI1*) F-box protein enhances interactions between *COI1* and jasmonate ZIM-domain (*JAZ*) proteins, resulting in the degradation of *JAZ* proteins via an ubiquitin/26S proteasome-mediated process. This leads to a release of the transcription factor *MYC2*, the expression of downstream JA responsive genes, and physiological responses (Chini et al., 2007; Dombrecht et al., 2007; Thines et al., 2007; Yan et al., 2009). *JAR1* plays an important role in the regulation of JA responses in *Arabidopsis*. *JAR1* homologs have been found in other plant species, including tomato, tobacco, and rice (Kang et al., 2006; Wang et al., 2007; Riemann et al., 2008; Suza et al., 2010). Kang et al. (2006) reported that silencing the *JAR1* homologous gene *Nicotiana tabacum JAR4*, via virus-induced gene silencing, decreased JA-Ile levels and increased plant susceptibility to *Manduca sexta* attack. *JAR1* and its homologs may thus participate in the control of biotic invaders.

In this study, we isolated *Solanum lycopersicum* jasmonate-resistant 1-like 1 (*SIJRL1*) encoding a 577 amino-acid protein with 99.3% amino-acid identity with *Solanum lycopersicum* jasmonate-resistant 1 (*SIJAR1*) in tomato (Suza et al., 2010). Moreover, we generated transgenic tomato lines harboring a *SIJRL1* gene construct driven by a glucocorticoid-inducible promoter. The transcripts of *SIJRL1* were greatly increased in the roots of transgenic

plants treated with dexamethasone (DEX). High levels of *SIJRL1* in transgenic tomato resulted in delayed colonization of *R. solanacearum* Pss4 and delayed the development of wilt symptoms after bacterial inoculation. Thus, JA interferes with the virulence of the wilt bacteria.

MATERIALS AND METHODS

Plant materials and growth conditions

Tomato (*Solanum lycopersicum* Mill.) cultivar CL5915 was used for gene isolation and transformation. Seeds were kindly provided by AVRDC-The World Vegetable Center, Tainan, Taiwan. Seeds were surface sterilized with 1% NaOCl by standard procedures and then germinated on Murashige and Skoog (MS) basal medium supplemented with 1% sucrose with a 16-h photoperiod at 26°C.

SIJRL1 cloning from tomato cultivar CL5915

To isolate the tomato homolog of *AtFIN219/JAR1* gene, we searched the tomato expressed sequence tag (EST) library (Computational Biology and Functional Genomics Laboratory, <http://compbio.dfci.harvard.edu/tgi/>) using the sequence for the *AtFIN219/JAR1* cDNA. The EST clones TC129298, TC124338 and TC124339 showed high similarity. These 3 EST clones can be assembled into a full-length clone that is predicted to encode a 577 amino acid protein, named *SIJRL1*. The cDNA fragment of *SIJRL1* was amplified with use of the primers 5'-TCTAGAATGAAGATGATGGTGGAAAATATTG-3' and 5'-ACATTGGGACGACCGGTAAGATCT-3' with the cDNA template derived from the reverse transcription of total RNAs isolated from the roots of tomato cultivar CL5915.

DNA and RNA gel blot analyses of *SIJRL1* in tomato line CL5915

To detect the copy number of *SIJRL1* in CL5915 or transgenic tomato lines, genomic DNA was isolated from tomato seedlings and digested with various restriction enzymes at 37°C overnight (Hsieh et al., 1996). The resulting DNA was then separated on a 0.7% agarose gel and blotted onto a charged nylon membrane (Roche). The gene-specific fragment of *SIJRL1* in CL5915 or the full-length fragment of hygromycin phosphotransferase (*HptII*) for transgenic tomato lines was used as a probe for DNA gel blot analysis. The probes were labeled with DIG-dUTP by PCR amplification with the primers 5'-TGTCTGGACAAATCGTTCCT-3' and 5'-TTGGACCAACACATCTAGGG-3' for *SIJRL1* and 5'-GTGCTTGACATTGGGGAGTT-3' and 5'-TTATGCTCCAGCGGTTGTAG-3' for the *HptII* fragment. To detect expression patterns, total RNA was isolated from various tissues of tomato CL5915 plants or from transgenic tomato lines induced by 5 µM dexamethasone (DEX), then subjected to RNA gel blot analysis using a *SIJRL1*-specific probe. The results were then detected with the LAS-3000 imaging system (FujiFilm).

Plasmid constructs and transformation

The cDNA fragment of *SIJRL1* was amplified with the primers 5'-TCTAGAATGAAGATGATGGTGGAAAATATTG-3' and 5'-ACATTGGGACGACCGTAAGATCT-3' by RT-PCR with the template generated from the total RNA of tomato roots. The resulting cDNA fragment was cloned into yT&A vector (Yeastern Biotech. Co.) and named yT&A/*SIJRL1* for sequence verification. *SIJRL1* was then excised from yT&A/*SIJRL1* as a *XbaI* fragment and sub-cloned into the *pTA7002* vector (Aoyama and Chua, 1997) with a DEX-inducible promoter. The *pTA7002/SIJRL1* was then transformed into CL5915 via *Agrobacterium*-mediated transformation (Chan et al., 2005).

Infection with *R. solanacearum*

Transgenic seeds of the T1 generation were screened on MS medium supplemented with 20 µg/ml hygromycin for two weeks, and wild-type seeds were germinated on MS medium alone. The hygromycin-resistant T1 tomato plants and wild-type plants were transferred to 2-inch-diameter plastic pots containing sand, soil, rice husks, and compost (1:3:1:1) and incubated at 28°C under 16-h light/8-h dark for two weeks. The preparation of the inoculum *R. solanacearum* strain *Pss4* was described previously (Lin et al., 2008). Briefly, bacteria were grown on 523 medium (0.3 g/l MgSO₄·7H₂O, 2.0 g/l K₂HPO₄, 4.0 g/l yeast extract, 8.0 g/l casein hydrolysate, 10.0 g/l sucrose, 15g/l agar) supplemented with 50 mg/l TTC (2,3,5-triphenyltertrazolum chloride) at 28°C for 24 h, harvested and suspended in sterile water to adjust the concentration to 10⁸ colony

forming units/ml. Roots of four-week-old tomato plants were damaged with a knife, and bacteria inoculum was poured into pots. Inoculated tomato plants were incubated at 28°C to develop wilt symptoms. For the induction of transgenic *SIJRL1* expression, 5 µM DEX was added to each pot at the indicated time. As a control, 75 ml water was added to pots. Two biological experiments were performed, each experiment with three replicates, and each replicate involved ten plants.

Analysis of bacterial growth

To detect the propagation of bacteria, tissue 2 cm below the shoot apex of infected tomato plants was collected at different times and ground in sterile water. The extracts were then serially diluted with sterile water, and the titer of bacteria was analyzed on SM1 medium (100 mg/l polymyxin B sulfate, 20 mg/l tyrothricin, 5 mg/l chloramphenicol, 5 mg/l cycloheximide, 50 mg/l TTC, 5 mg/l crystal violet) at 28°C (Lin et al., 2008). Five plants were collected at each time, along with three bacterial growth replicates per plant.

RESULTS

Isolation of tomato *JAR1*-like gene, *SIJRL1*, from heat-tolerant tomato CL5915

Arabidopsis JAR1/FIN219 is a JA-conjugating enzyme responsible for producing JA-Ile (Staswick et al., 2002) and plays vital roles in far-red light and JA signaling integration (Hsieh et al., 2000; Chen et al., 2007). To obtain tomato homologs of JAR1/FIN219, we searched the to-

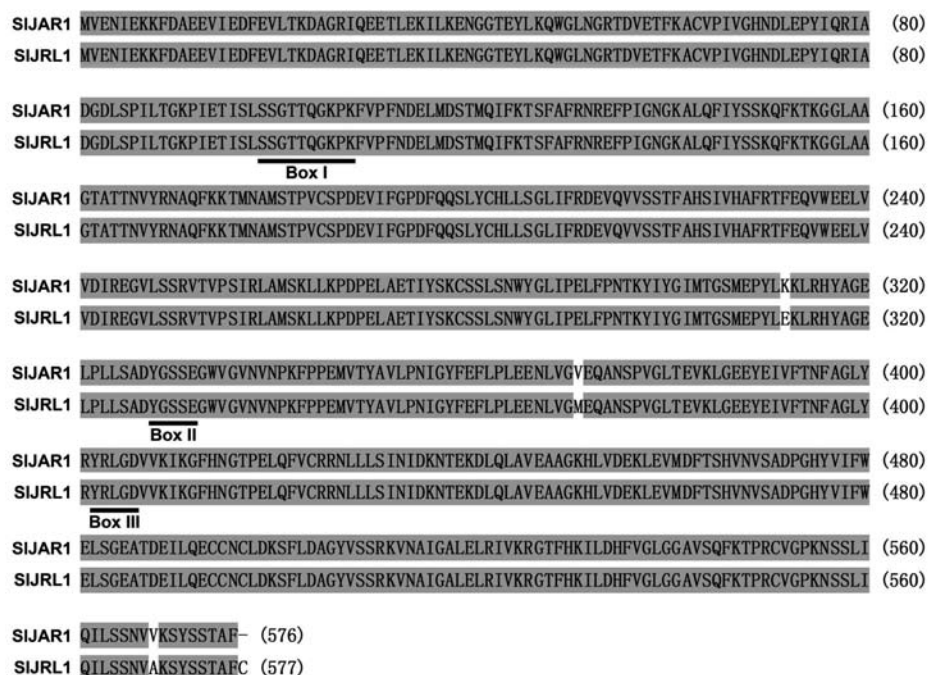


Figure 1. Alignment of *Solanum lycopersicum* jasmonate-resistant 1 (*SIJAR1*) and *SIJAR1*-like 1 (*SIJRL1*) amino acid residues. The solid lines indicate adenylate-forming domains, box I, box II, and box III. Different amino acid residues between *SIJAR1* and *SIJRL1* are not shaded.

mato EST library (Computational Biology and Functional Genomics Laboratory <http://compbio.dfci.harvard.edu/tgi/>) using the sequence for *AtFIN219/JAR1* cDNA, and found three overlapping EST clones that could be assembled into a full-length cDNA fragment. The cDNA clone corresponding to the tomato *JAR1-like* gene, named *SIJRL1*, was eventually obtained by RT-PCR with cDNA templates derived from total RNA of the heat-tolerant tomato line CL5915. *SIJRL1* encodes a 577 amino-acid polypeptide consisting of three adenylate-forming domains (Figure 1) that participate in ATP/AMP binding (Chang et al., 1997) and JA-Ile synthesis (Staswick and Tiryaki, 2004). Moreover, *SIJRL1* shared more than 99% amino acid identity with *SIJAR1*, with only three amino-acid differences between sequences (Figure 1). To further determine the copy number of *SIJRL1* in the tomato genome, we subjected genomic DNA digested with various restriction enzymes to DNA gel blot analysis using a gene-specific probe in the 3' end of *SIJRL1* cDNA (Figure 2A). Both *Bam*HI and *Hind*III restriction sites are absent from the coding region of *SIJRL1* cDNA (Figure 2A). We found 6~8 bands under highly stringent conditions among different enzyme-digested DNA samples (Figure 2B), which suggests that the tomato genome may contain multiple copies of *SIJRL1* gene. An assay of tissue-specific expression patterns revealed *SIJRL1* transcripts in all tissues examined, with the greatest abundance in leaves and the least in roots (Figure 2C).

Induction of *SIJRL1* expression in transgenic tomato treated with DEX

To further understand the functions of *SIJRL1* in tomato, we tried to introduce an overexpression or RNA interference construct of *SIJRL1* into the heat-tolerant tomato line CL5915 by *Agrobacterium*-mediated transformation; however, we failed to obtain any putative transgenic tomato plants (data not shown), implying that *SIJRL1* may have an essential role in tomato. We further generated transgenic tomato plants harboring the *SIJRL1* gene driven by a glucocorticoid-inducible promoter (Figure 3A) induced by the application of DEX (Aoyama and Chua, 1997). Transgenic tomatoes were screened on medium supplemented with hygromycin, and the insertion number of the transgenic *SIJRL1* gene was examined by DNA gel blot analysis with the full-length *HPTII* used as a probe. Only a single insertion was observed in the test lines (Figure 3B).

To understand the expression patterns of *SIJRL1* in transgenic tomatoes, the roots of the transgenic line 1

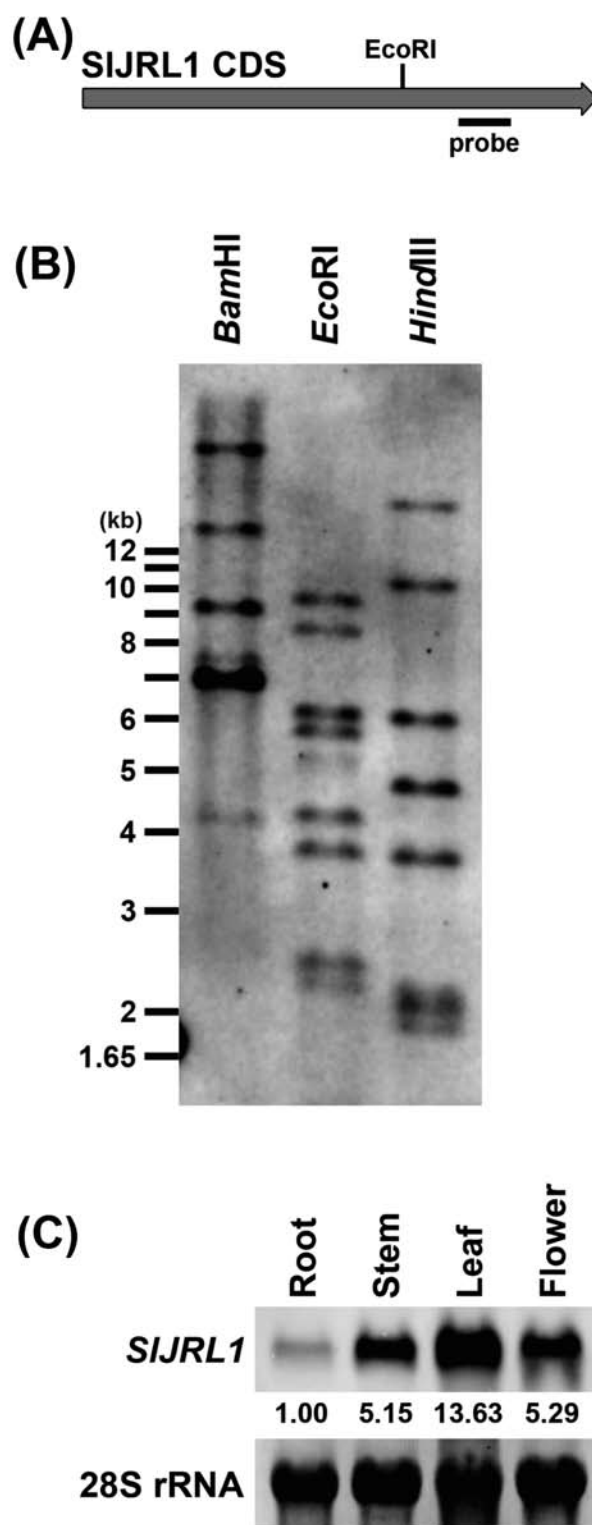


Figure 2. *SIJRL1* has multiple copies in the tomato genome and high expression in aerial tissues. (A) Schematic diagram of the coding region of *SIJRL1* cDNA. The specific probe used for DNA gel blot analysis shown in (B) is indicated below the diagram. One *Eco*RI site is present in the coding region of *SIJRL1* Cdna; (B) DNA gel blot analysis of the *SIJRL1* gene in the tomato genome. Genomic DNA isolated from heat-tolerant tomato CL5915 was digested with various restriction enzymes shown above the blot and hybridized with a dig-labeled specific DNA probe corresponding to the C-terminal region of *SIJRL1*. The number shown at the left of the blot indicates 1 kb plus DNA ladder (Invitrogen); (C) RNA gel blot analysis of tissue-specific expression patterns of *SIJRL1* in tomato. Total RNA isolated from different tissues of tomato CL5915 was analyzed for *SIJRL1* expression. Each lane was loaded with 10 μ g total RNA. 28S rRNA stained with methylene blue was a loading control. The numbers below the blot represent relative expression ratios normalized to the levels of 28S rRNA expression; the level of *SIJRL* transcripts in roots was arbitrarily set to 1.

were collected before and after DEX treatments and used for RNA extraction, followed by RNA gel blot analyses. *SIJRL1* transcripts were detected in the roots of transgenic line 1 before DEX treatments (Figure 4A), indicating the basal levels of endogenous *SIJRL1* expression. Further, the expression of *SIJRL1* was enhanced and peaked at two days after 5 μ M DEX treatment, then was greatly reduced at four days (Figure 4A). The transgenic tomato lines 11 and 16 also showed significant induction of *SIJRL1* expression with DEX treatment (Figure 4B). These transgenic tomato lines were thus useful for further functional studies.

Induction of *SIJRL1* expression could delay the colonization of *R. solanacearum* in transgenic tomato

Because *Arabidopsis* JAR1/FIN219 has a role in pathogen-triggered defense responses (Staswick and Tiryaki,

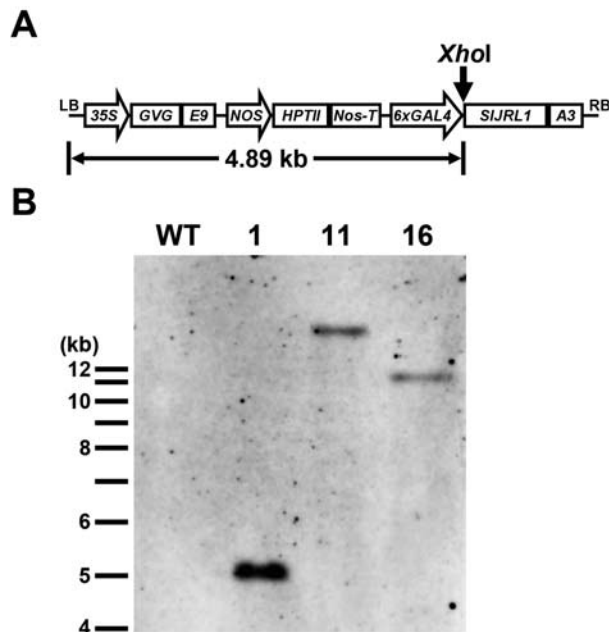


Figure 3. Transgenic tomato plants contain a single copy of the transgene *SIJRL1*. (A) Schematic diagram of the *SIJRL1* expression construct driven by a glucocorticoid-inducible promoter. LB and RB are left and right border, respectively. 35S: cauliflower mosaic virus 35S promoter. GVG: artificial fusion protein of GAL4 DNA binding domain-VP16 transactivating domain-glucocorticoid receptor domain. E9: terminator of the pea ribulose biphosphate carboxylase small subunit rbc3-E9. NOS: nopaline synthase promoter. HPTII: hygromycin phosphotransferase. 6xGAL4: six copies of the GAL4 upstream activating sequence. Nos-T: terminator of nopaline synthase. A3: terminator of pea rbc3-3A. The arrow indicates *XhoI* restriction site; (B) DNA gel blot analysis of the transgene *SIJRL1* in transgenic tomatoes. The genomic DNAs of wild-type (WT) and transgenic tomato lines 1, 11, and 16 were digested with *XhoI* overnight, subjected to DNA gel blot analysis, and hybridized with *NPTII* dig-labeled probe. The numbers on the left are 1 kb plus DNA ladder (Invitrogen).

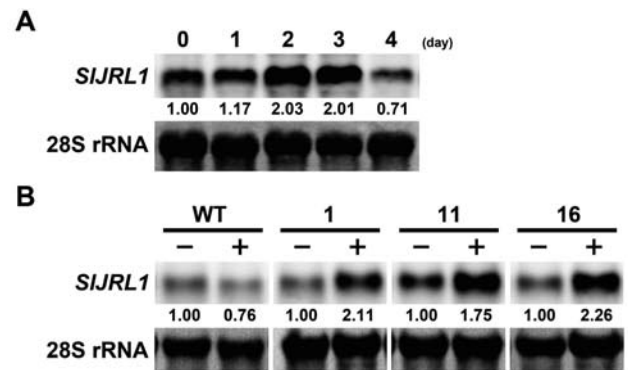


Figure 4. *SIJRL1* shows high levels of expression in transgenic tomatoes. (A) RNA gel blot analysis of *SIJRL1* expression at different times for transgenic tomato line 1 under dexamethasone (DEX) treatment. Total RNA was isolated from the roots of transgenic tomato line 1 with induction by 5 μ M DEX treatment, grown under white light, and used for RNA gel blot analysis. The probe used is a dig-labeled *SIJRL1* cDNA fragment. 28S rRNA was a loading control. The numbers below the blot represent relative expression ratios normalized to the levels of 28S rRNA expression; the level of *SIJRL1* transcripts at time 0 was arbitrarily set to 1; (B) RNA gel blot analysis of *SIJRL1* expression in different lines of transgenic tomato under DEX treatment. Total RNA was isolated from the roots of different transgenic lines of tomatoes at 2 days after 5 μ M DEX (+) or water (-) treatment and subjected to RNA gel blot analysis. WT: wild-type; 1, 11, and 16: different transgenic tomato lines. The numbers below the blot represent relative expression ratios normalized to the levels of 28S rRNA expression; the level of *SIJRL1* transcripts in various samples with water treatment (-) was arbitrarily set to 1.

2004; Browse, 2009), we wondered whether *SIJRL1* participates in the resistance responses against *R. solanacearum* infection. Wild-type and transgenic tomatoes inoculated with *R. solanacearum* showed similar wilt disease symptoms timing (Figure 5A). 40% showed wilt disease two days after infection (dai); almost all plants were wilted at 6 dai (Figure 5A). A similar trend was observed in wild-type plants treated with DEX (Figure 5B), implying that DEX treatment did not interfere with the virulence of *R. solanacearum* infection. In contrast, transgenic tomato treated with DEX at 2 dai showed no wilt symptoms (Figure 5B). Furthermore, with DEX treatment, 40% of wild-type plants showed wilt disease at 3 dai compared with less than 10% for transgenic plants. The proportion of wild-type and transgenic plants with wilt disease substantially differed at 4 dai, with fewer differences at later stages of infection (Figure 5B). Induction of *SIJRL1* expression can thus delay the development of wilt symptoms in transgenic tomatoes after *R. solanacearum* infection.

Wilt symptoms in plants infected with *R. solanacearum* are caused by the colonization of *R. solanacearum* in the xylem tissues, thus interrupting the translocation of water to leaves (Chan et al., 2005). To further determine whether *SIJRL1* is involved in the disruption of *R. solanacearum* colonization, we examined the titer of *R. solanacearum* in the stem 2 cm below the shoot tip. The titer of *R. solan-*

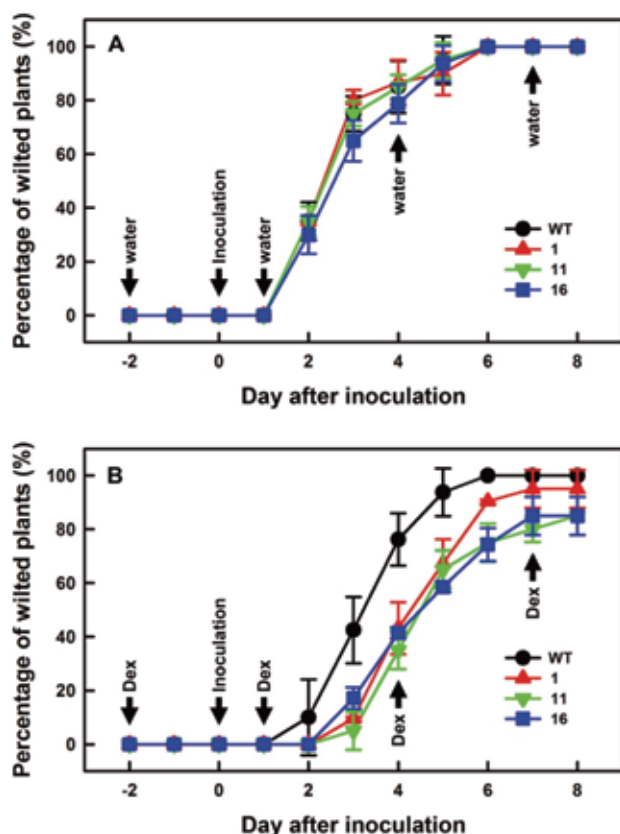


Figure 5. Induction of *SLJRL1* expression results in delayed development of bacterial wilt symptoms in tomato. Wild-type and transgenic tomato lines were inoculated with *Ralstonia* and induced by water (A) or 5 μ M DEX (B). The percentage of wilted tomato plants under white light was recorded. Arrows indicate the times of *Ralstonia* inoculation and DEX induction.

acearum isolated from wild-type and transgenic tomatoes with water treatment remained largely the same, increased rapidly at 1 dai and peaked at 3 dai (Figure 6A). Similar results were observed in wild-type plants treated with DEX (Figure 6B). In contrast, transgenic tomatoes treated with DEX showed no *R. solanacearum* colonization at 1 dai; the titer of *R. solanacearum* was detected at 2 dai and peaked at 4 dai (Figure 6B). High levels of *SLJRL1* expression can thus delay the colonization of *R. solanacearum* in xylem tissues and postpone the development of wilt symptoms.

DISCUSSION

Here, we report on the isolation and characterization of the tomato homolog *SLJRL1* of *Arabidopsis JAR1/FIN219*. The tomato genome contains multiple copies of *SLJRL1*. *SLJRL1* shows high expression in aerial tissues, including stems, leaves and flowers, especially leaves. We generated transgenic tomato lines containing a DEX-inducible *SLJRL1* construct, and DEX-induced *SLJRL1* expression could delay the symptoms of bacterial wilt disease. Thus, JA can interfere with the detrimental damage caused by *Ralstonia* infection.

Previous reports showed that *jar1* mutants and *JAR1*-silenced plants contained reduced levels of JA-Ile (Staswick and Tiryaki, 2004; Kang et al., 2006; Wang et al., 2007; Suza et al., 2010). *Arabidopsis jar1-1* and *jar1-3* mutants were mutated in box I and box II motifs, respectively, and showed insensitive phenotypes to methyl JA (Staswick et al., 2002). Moreover, both *jar1-1* and *jar1-11* (a null mutant) appear to have equal levels of JA-Ile in unwounded and wounded leaves, suggesting that the *jar1-1* protein seems to have no conjugating activity (Staswick and Tiryaki, 2004; Suza and Staswick, 2007). In addition, *JAR1* overexpression in *jar1-1* could restore the JA-Ile to the wild-type level and the JA responses (Staswick and Tiryaki, 2004). All these data support the importance of three adenylate-forming boxes in *JAR1* in mediating the conjugation of JA and Ile. At present, five homologous *JAR1* proteins were annotated in four species, including *Arabidopsis*, tomato, tobacco, and rice (Staswick et al., 2002; Kang et al., 2006; Wang et al., 2007; Riemann et al., 2008; Suza et al., 2010). Except for *Oryza sativa JAR1*, other *JAR1* homologs are involved in the generation of

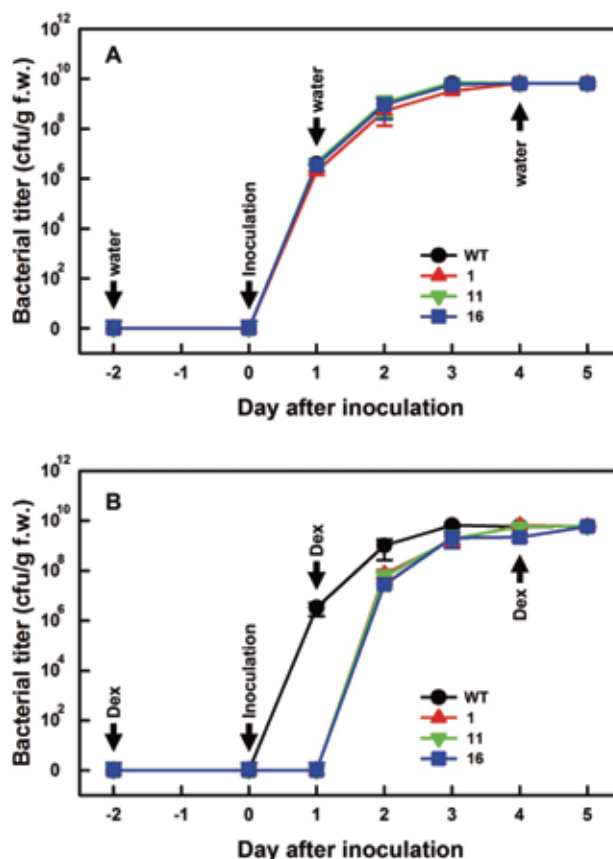


Figure 6. Induction of *SLJRL1* expression results in a reduction of the *Ralstonia* titer in the stems of transgenic tomatoes. Wild type and transgenic tomato lines were inoculated with *Ralstonia* and induced by water (A) or 5 μ M DEX (B). The titer of bacteria in the stems of wild type and transgenic tomatoes under white light was recorded. Arrows indicate the times of *Ralstonia* inoculation and DEX induction.

JA-Ile. Here, we identified *SIJRL1* as a new homolog of the *JAR1* gene in tomato. Three adenylate-forming boxes are conserved among 6 JAR1 homologs (Figure S1). Especially in the *Solanaceae* family, no residues of adenylate-forming boxes are changed. *Nicotiana tabacum* JAR4 (NtJAR4) shows 86.9% amino-acid identity with NtJAR6, which suggests that they are involved in JA-Ile synthesis (Kang et al., 2006; Wang et al., 2007). In contrast, *SIJRL1* and *SIJAR1* share 99.3% identity. Thus, *SIJRL1* may have conjugating activity for JA-Ile.

High levels of *SIJRL1* expression in transgenic tomatoes resulted in a delayed development of wilt symptoms after inoculation with *R. solanacearum* (Figure 5), which is similar to that observed in previous reports (Yang et al., 2007; Yang et al., 2008). *LJAMP1* and *LJAMP2* with antimicrobial activities were introduced into tobacco, thus leading to delayed development of wilt symptoms caused by *R. solanacearum* (Yang et al., 2007; Yang et al., 2008). In addition, the overexpression of *Arabidopsis thionin 2.1* (*AtTH2.1*) in tomato substantially reduced the population of *R. solanacearum* in stem tissues and further delayed the development of wilt symptoms (Chan et al., 2005). Similarly, overexpression of sweet pepper ferredoxin-I protein reduced colonization and wilt disease (Huang et al., 2007). Thus, ectopic expression of these genes inhibited the intrusion and growth of bacteria and interfered with the upward movement of bacteria from root to stem, which further diminished the development of wilt symptoms (Wang et al., 2000; Wang and Lin, 2003; Chan et al., 2005). Here, our results suggest a similar mechanism against *R. solanacearum* infection conferred by the DEX-induced *SIJRL1* in transgenic tomatoes, which led to the 1 dai abolishment of *R. solanacearum* population in transgenic tomatoes (Figure 6B). Transgenic tomatoes harboring high levels of *SIJRL1* expression can thus delay bacteria wilt disease and reduce the bacteria population in stems.

In addition to being antibacterial proteins, ET and SA are defense phytohormones against *R. solanacearum* infection in plants (Deslandes et al., 2002; Hirsch et al., 2002; Deslandes et al., 2003; Valls et al., 2006; Qiao et al., 2009; Stepanova and Alonso, 2009). *Tomato stress responsive factor 1* (*TSRF1*), an ET response factor, was upregulated by ET, SA, and *R. solanacearum* infection. *TSRF1* protein directly bound to the GCC box, thus activating the expression of *PR* genes to enhance resistance to *R. solanacearum* infection in tobacco (Zhang et al., 2004). Similar evidence was found with barley *HvRAF* and soybean *GmERF3*. *HvRAF* and *GmERF3* belong to the ERF family, and directly activate several *PR* genes to confer protection. The expression of *HvRAF* and *GmERF3* was increased by ET and SA, and induced by JA (Jung et al., 2007; Zhang et al., 2009). Hirsch et al. (2002) reported that the *jar1-1* mutant without conjugating activities for JA-Ile was more susceptible to *R. solanacearum* infection, implying that JAR1 may be involved in the defense response in *Arabidopsis* against *R. solanacearum* infection. The *coi1* mutant loss-of-function in tomato resulted in the suppression of JA-



Figure S1. Alignment of SIJRL1-related proteins from different plant species. The solid lines indicate adenylate-forming domains. AtJAR1: *Arabidopsis thaliana* jasmonate resistance 1. OsJAR1: *Oryza sativa* JAR1. NtJAR4: *Nicotiana tabacum* JAR4. NtJAR6: *Nicotiana tabacum* JAR6. SIJAR1: *Solanum lycopersicum* JAR1. SIJRL1: *Solanum lycopersicum* JAR1-Like 1.

responsive gene expression, including those involved in JA biosynthesis, signaling, and proteinase inhibitor proteins, leading to greater susceptibility to mites (Li et al., 2004). Moreover, the biocontrol agent *Pythium oligandrum* (PO) colonized the rhizosphere of tomato to suppress wilt disease caused by *R. solanacearum* infection, but tomato plants became susceptible in the *jail-1* mutant (mutated in the *COI1* gene) treated with PO homogenate (Hase et al., 2008). The PO homogenate also induced the expression of *PR-6* via *COI1*-dependent signaling, but not SA signaling, and further promoted tomato resistance against *R. solan-*

acearum infection (Hase et al., 2008). Thus, enhancing JA signaling provided protection against *R. solanacearum* infection. Furthermore, JA-Ile is the active form of the JA hormone and is required for plant defense against insect and pathogen invasion (see reviews in Browse, 2009). Our results indicated that a high *SIJRL1* expression may promote resistance against *R. solanacearum* infection (Figures 4-6). Thus, *SIJRL1* reinforces protection against bacterial wilt disease via JA signaling in tomato.

Of note, our results and other evidence, such as PO-treated plants, show a delay in bacterial wilt disease with infection (Figures 5 and 6; Hase et al., 2008). Compared with our results, PO treatment delayed wilt symptoms for about one day. PO-treated tomato may confer more resistance against *R. solanacearum* infection due to the purified elicitors (POD-1 and POD-2) from the PO cell wall proteins and PO homogenate, which increase ET induce ET- and JA-related PR genes expression to promote protection against pathogens (Takenaka et al., 2003; Takenaka et al., 2006; Takenaka et al., 2008). Moreover, JA and ET synergistically participate in the plant defense responses to many pathogens (for reviews, see Kunkel and Brooks, 2002; Van Loon et al., 2006). However, null or silenced JAR1-homolog mutants still contain certain levels of JA-Ile, suggesting that another component for JA-Ile biosynthesis in plants regulating JA signaling may exist (Staswick and Tiriyaki, 2004; Suza and Staswick, 2007; Kang et al., 2006; Wang et al., 2007; Suza et al., 2010). This speculation is supported by the finding of two isoforms, NaJAR4 and NaJAR6, for the biosynthesis of JA-Ile in *Nicotiana attenuata* (Wang et al., 2007). In addition, we found multiple copies of SIJAR1 homologs in tomato (Figure 2A). Although SIJRL1 and SIJAR1 share 99% amino acid identity, *SIJRL1* transcripts are abundant in stems, leaves, and flowers, but much less so in roots (Figure 2C). However, *SIJAR1* expression is abundant in roots and opened flowers, but not in leaves (Suza et al., 2010). Both genes exhibit tissue-specific expression patterns, which might be determined by specific transcription factors binding to the cis-elements present in the promoter regions of individual genes. This suggests that SIJRL1 and SIJAR1 may have different functions in JA signaling. In addition, because of the multiple copies of *SIJAR1* homologs in tomato, an increase of only *SIJRL1* expression may not confer enough JA-Ile levels to trigger bacterial wilt disease resistance. Alternatively, *SIJRL1* induction may restrict the availability of the substrate in the aboveground tissues because *SIJRL1* showed less expression in roots, leading to delayed and reduced bacterial wilt disease symptoms. Therefore, examining the effect of *SIJAR1* induction on the infection or colonization of *Ralstonia* in tomato is of interest, as are the unique molecular mechanisms in *SIJAR1* and *SIJRL1* that underly bacterial wilt disease resistance in tomato.

Acknowledgements. We thank Chiu-Ping Cheng, Institute of Plant Biology, National Taiwan University, Taiwan, for the kind gift of the bacterial strain *Ralstonia solanacearum*. This work was supported by a grant from Coun-

cil of Agriculture Executive Yuan (95Agriscience-6.1.3-crop-Z1(4)-2), Taiwan.

LITERATURE CITED

- Adhikari, T.B. and R.C. Basnyat. 1998. Effect of crop rotation and cultivar resistance on bacterial wilt of tomato in Nepal. *Can. J. Plant Pathol.* **20**: 283-287.
- Aoyama, T. and N.-H. Chua. 1997. A glucocorticoid-mediated transcriptional induction system in transgenic plants. *Plant J.* **11**: 605-612.
- Browse, J. 2009. Jasmonate Passes Muster: A receptor and targets for the defense hormone. *Annu. Rev. Plant Biol.* **60**: 183-205.
- Chan, Y.-L., V. Prasad, Sanjaya, K.H. Chen, P.C. Liu, M.-T. Chan, and C.-P. Cheng. 2005. Transgenic tomato plants expressing an Arabidopsis thionin (Thi2.1) driven by fruit-inactive promoter battle against phytopathogenic attack. *Planta* **221**: 386-393.
- Chang, K.-H., H. Xiang, and D. Dunaway-Mariano. 1997. Acyl-adenylate motif of the acyl-adenylate/thioester-forming enzyme superfamily: A site-directed mutagenesis study with the *Pseudomonas* sp. strain CBS3 4-chlorobenzoate:coenzyme A ligase. *Biochemistry* **36**: 15650-15659.
- Chen, I.C., I.C. Huang, M.J. Liu, Z.G. Wang, S.S. Chung, and H.L. Hsieh. 2007. Glutathione S-transferase interacting with far-red insensitive 219 is involved in phytochrome A-mediated signaling in Arabidopsis. *Plant Physiol.* **143**: 1189-1202.
- Chini, A., S. Fonseca, G. Fernandez, B. Adie, J.M. Chico, O. Lorenzo, G. Garcia-Casado, I. Lopez-Vidriero, F.M. Lozano, M.R. Ponce, J.L. Micol, and R. Solano. 2007. The JAZ family of repressors is the missing link in jasmonate signaling. *Nature* **448**: 666-671.
- Deslandes, L., J. Olivier, F. Theulières, J. Hirsch, D.X. Feng, P. Bittner-Eddy, J. Beynon, and Y. Marco. 2002. Resistance to *Ralstonia solanacearum* in *Arabidopsis thaliana* is conferred by the recessive *RRS1-R* gene, a member of a novel family of resistance genes. *Proc. Natl. Acad. Sci. USA* **99**: 2404-2409.
- Deslandes, L., J. Olivier, N. Peeters, D.X. Feng, M. Khounlotham, C. Boucher, I. Somssich, S. Genin, and Y. Marco. 2003. Physical interaction between RRS1-R, a protein conferring resistance to bacterial wilt, and PopP2, a type III effector targeted to the plant nucleus. *Proc. Natl. Acad. Sci. USA* **100**: 8024-8029.
- Dombrecht, B., G.P. Xue, S.J. Sprague, J.A. Kirkegaard, J.J. Ross, J.B. Reid, G.P. Fitt, N. Sewelam, P.M. Schenk, J.M. Manners, and K. Kazan. 2007. MYC2 differentially modulates diverse jasmonate-dependent functions in *Arabidopsis*. *Plant Cell* **19**: 2225-2245.
- Genin, S. and C. Boucher. 2004. Lessons learned from the genome analysis of *Ralstonia solanacearum*. *Annu. Rev. Phytopathol.* **42**: 107-134.

- Guo, J.-H., H.-Y. Qi, Y.-H. Guo, H.-L. Ge, L.-Y. Gong, L.-X. Zhang, and P.-H. Sun. 2004. Biocontrol of tomato wilt by plant growth-promoting rhizobacteria. *Biol. Control* **29**: 66-72.
- Hase, S., S. Takahashi, S. Takenaka, K. Nakaho, T. Arie, S. Seo, Y. Ohashi, and H. Takahashi. 2008. Involvement of jasmonic acid signalling in bacterial wilt disease resistance induced by biocontrol agent *Pythium oligandrum* in tomato. *Plant Pathol.* **57**: 870-876.
- Hayward, A.C. 1991. Biology and epidemiology of bacterial wilt caused by *Pseudomonas solanacearum*. *Annu. Rev. Phytopathol.* **29**: 65-87.
- Hirsch, J., L. Deslandes, D.X. Feng, C. Balagué, and Y. Marco. 2002. Delayed symptom development in *ein2-1*, an Arabidopsis ethylene-insensitive mutant, in response to bacterial wilt caused by *Ralstonia solanacearum*. *Phytopathology* **92**: 1142-1148.
- Hsieh, H.-L., C.-G. Tong, C. Thomas, and S.J. Roux. 1996. Light-modulated abundance of an mRNA encoding a calmodulin-regulated, chromatin-associated NTPase in pea. *Plant Mol. Biol.* **30**: 135-147.
- Hsieh, H.-L., H. Okamoto, M. Wang, L.-H. Ang, M. Matsui, H. Goodman, and X.W. Deng. 2000. FIN219, an auxin-regulated gene, defines a link between phytochrome A and the downstream regulator COP1 in light control of Arabidopsis development. *Gene Dev.* **14**: 1958-1970.
- Huang, H.-E., C.-A. Liu, M.-J. Lee, C.-G. Kuo, H.-M. Chen, M.-J. Ger, Y.-C. Tsai, Y.-R. Chen, M.-K. Lin, and T.-Y. Feng. 2007. Resistance enhancement of transgenic tomato to bacterial pathogens by the heterologous expression of sweet pepper ferredoxin-I protein. *Phytopathology* **97**: 900-906.
- Jung, J., S. Won, S. Suh, H. Kim, R. Wing, Y. Jeong, I. Hwang, and M. Kim. 2007. The barley ERF-type transcription factor HvRAF confers enhanced pathogen resistance and salt tolerance in *Arabidopsis*. *Planta* **225**: 575-588.
- Kang, J.-H., L. Wang, A. Giri, and I.T. Baldwin. 2006. Silencing threonine deaminase and JAR4 in nicotiana attenuata impairs jasmonic acid-isoleucine-mediated defenses against *manduca sexta*. *Plant Cell* **18**: 3303-3320.
- Katsir, L., H.S. Chung, A.J.K. Koo, and G.A. Howe. 2008. Jasmonate signaling: a conserved mechanism of hormone sensing. *Curr. Opin. Plant Biol.* **11**: 428-435.
- Kunkel, B.N. and D.M. Brooks. 2002. Cross talk between signaling pathways in pathogen defense. *Curr. Opin. Plant Biol.* **5**: 325-331.
- Li, L., Y. Zhao, B.C. McCaig, B.A. Wingerd, J. Wang, M.E. Whalon, E. Pichersky, and G.A. Howe. 2004. The tomato homolog of CORONATINE-INSENSITIVE1 is required for the maternal control of seed maturation, jasmonate-signaled defense responses, and glandular trichome development. *Plant Cell* **16**: 126-143.
- Lin, Y.-M., I.-C. Chou, J.-F. Wang, F.-I. Ho, Y.-J. Chu, P.-C. Huang, D.-K. Lu, H.-L. Shen, M. Elbaz, S.-M. Huang, and C.-P. Cheng. 2008. Transposon mutagenesis reveals differential pathogenesis of *Ralstonia solanacearum* on tomato and *Arabidopsis*. *Mol. Plant-Microbe Interact.* **21**: 1261-1270.
- Qiao, H., K.N. Chang, J. Yazaki, and J.R. Ecker. 2009. Interplay between ethylene, ETP1/ETP2 F-box proteins, and degradation of EIN2 triggers ethylene responses in *Arabidopsis*. *Gene Dev.* **23**: 512-521.
- Riemann, M., M. Riemann, and M. Takano. 2008. Rice JASMONATE RESISTANT 1 is involved in phytochrome and jasmonate signalling. *Plant Cell Environ.* **31**: 783-792.
- Schell, M.A. 2000. Control of virulence and pathogenicity genes of *Ralstonia solanacearum* by an elaborate sensory network. *Annu. Rev. Phytopathol.* **38**: 263-292.
- Staswick, P.E. and I. Tiryaki. 2004. The oxylipin signal jasmonic acid is activated by an enzyme that conjugates it to isoleucine in *Arabidopsis*. *Plant Cell* **16**: 2117-2127.
- Staswick, P.E., I. Tiryaki, and M.L. Rowe. 2002. Jasmonate response locus JAR1 and several related Arabidopsis genes encode enzymes of the firefly luciferase superfamily that show activity on jasmonic, salicylic, and indole-3-acetic acids in an assay for adenylation. *Plant Cell* **14**: 1405-1415.
- Stepanova, A.N. and J.M. Alonso. 2009. Ethylene signaling and response: where different regulatory modules meet. *Curr. Opin. Plant Biol.* **12**: 548-555.
- Suza, W. and P. Staswick. 2008. The role of JAR1 in jasmonoyl-isoleucine production during Arabidopsis wound response. *Planta* **227**: 1221-1232.
- Suza, W., M. Rowe, M. Hamberg, and P. Staswick. 2010. A tomato enzyme synthesizes (+)-7-iso-jasmonoyl-l-isoleucine in wounded leaves. *Planta* **231**: 717-728.
- Takenaka, S., Z. Nishio, and Y. Nakamura. 2003. Induction of defense reactions in sugar beet and wheat by treatment with cell wall protein fractions from the mycoparasite *Pythium oligandrum*. *Phytopathology* **93**: 1228-1232.
- Takenaka, S., Y. Nakamura, T. Kono, H. Sekiguchi, A. Masunaka, and H. Takahashi. 2006. Novel elicitor-like proteins isolated from the cell wall of the biocontrol agent *Pythium oligandrum* induce defence-related genes in sugar beet. *Mol. Plant Pathol.* **7**: 325-339.
- Thines, B., L. Katsir, M. Melotto, Y. Niu, A. Mandaokar, G. Liu, K. Nomura, S.Y. He, G.A. Howe, and J. Browse. 2007. JAZ repressor proteins are targets of the SCF^{COI1} complex during jasmonate signalling. *Nature* **448**: 661-665.
- Thoquet, P., J. Olivier, C. Sperisen, P. Rogowsky, H. Laterrot, and N. Grimsley. 1996. Quantitative trait loci determining resistance to bacterial wilt in tomato cultivar hawaii7996. *Mol. Plant-Microbe Interact.* **9**: 826-836.
- Valls, M., S. Genin, and C. Boucher. 2006. Integrated regulation of the type III secretion system and other virulence determinants in *Ralstonia solanacearum*. *PLoS Pathog.* **2**: 798-807.
- Van Loon, L.C., M. Rep, and C.M.J. Pieterse. 2006. Significance of inducible defense-related proteins in infected plants. *Annu. Rev. Phytopathol.* **44**: 135-162.
- Wang, J.-F., J. Olivier, P. Thoquet, B. Mangin, L. Sauviac, and N.H. Grimsley. 2000. Resistance of tomato line Hawaii7996

- to *Ralstonia solanacearum* pss4 in Taiwan is controlled mainly by a major strain-specific locus. Mol. Plant-Microbe Interact. **13**: 6-13.
- Wang, L., R. Halitschke, J.-H. Kang, A. Berg, F. Harnisch, and I. Baldwin. 2007. Independently silencing two JAR family members impairs levels of trypsin proteinase inhibitors but not nicotine. Planta **226**: 159-167.
- Xue, Q.-Y., Y. Chen, S.-M. Li, L.-F. Chen, G.-C. Ding, D.-W. Guo, and J.-H. Guo. 2009. Evaluation of the strains of Acinetobacter and Enterobacter as potential biocontrol agents against Ralstonia wilt of tomato. Biol. Control **48**: 252-258.
- Yan, J., C. Zhang, M. Gu, Z. Bai, W. Zhang, T. Qi, Z. Cheng, W. Peng, H. Luo, F. Nan, Z. Wang, and D. Xie. 2009. The Arabidopsis CORONATINE INSENSITIVE1 protein is a jasmonate receptor. Plant Cell **21**: 2220-2236.
- Yang, X., Y. Xiao, X. Wang, and Y. Pei. 2007. Expression of a novel small antimicrobial protein from the seeds of Motherwort (*Leonurus japonicus*) confers disease resistance in tobacco. Appl. Environ. Microb. **73**: 939-946.
- Yang, X., X. Wang, X. Li, B. Zhang, Y. Xiao, D. Li, C. Xie, and Y. Pei. 2008. Characterization and expression of an nsLTPs-like antimicrobial protein gene from motherwort. Plant Cell Rep. **27**: 759-766.
- Zhang, G., M. Chen, L. Li, Z. Xu, X. Chen, J. Guo, and Y. Ma. 2009. Overexpression of the soybean GmERF3 gene, an AP2/ERF type transcription factor for increased tolerances to salt, drought, and diseases in transgenic tobacco. J. Exp. Bot. **60**: 3781-3796.
- Zhang, H., D. Zhang, J. Chen, Y. Yang, Z. Huang, D. Huang, X.-C. Wang, and R. Huang. 2004. Tomato stress-responsive factor TSRF1 interacts with ethylene responsive element GCC box and regulates pathogen resistance to *Ralstonia solanacearum*. Plant Mol. Biol. **55**: 825-834.

轉殖番茄中 *Jasmonate-Resistant 1-like 1 (SJRL1)* 基因的誘導表現能夠延遲番茄青枯菌的菌落

王志恭 謝旭亮

國立台灣大學 植物科學研究所

番茄青枯病是一種非常普遍和廣泛的疾病，主要是由青枯菌引起，特別是在炎熱和潮濕的環境下更為嚴重；於自然環境中，因為寄主抗性的不穩定，以及青枯菌種類與變異性極大，至目前為止，並無良好的防治方式；因此，番茄抵禦青枯菌的分子機制的瞭解仍然有限。本篇報導由番茄釣取到番茄 *SIJARI* 同源性基因—*SIJRL1*，並且建立可誘導 *SIJRL1* 基因表現的番茄轉殖株。比對兩基因之蛋白質序列竟然高達 99 % 相同性；先前發現 *SIJARI* 於地下根部之基因表現量最高，相反地，*SIJRL1* 於地下部表現量最低，反而是地上部有較高的基因表現量；進一步利用南方氏點法分析，發現於 CL5915 番茄品系之基因體含有多個 *SIJRL1* 基因。進一步建構誘導啟動子驅動 *SIJRL1* 之載體，將其轉殖進番茄基因體中，利用 DEX 處理轉殖株，其誘導 *SIJRL1* 表現量於第二天即達到高峰；並利用此誘導方式，進行青枯菌感染，經 DEX 誘導之轉殖番茄可延緩萎凋病徵發生；並分析體內之青枯菌族群，發現轉殖番茄經由 DEX 誘導後，亦可減緩於莖頂處青枯菌累積之數量；由此可知，植物茉莉酸荷爾蒙具有參與番茄防禦青枯菌感染之功能。因此，本篇 *SIJRL1* 轉殖番茄之結果，可提供抵抗番茄青枯病之分子機制的瞭解。

關鍵詞：番茄青枯病；青枯菌；番茄 *SIJARI* 同源性基因—*SIJRL1*；茉莉酸荷爾蒙；番茄轉殖株。