European Journal of Plant Pathology **107:** 63–68, 2001.

Heritability of rhizobacteria-mediated induced systemic resistance and basal resistance in Arabidopsis

Jurriaan Ton, Sylke Davison, Leendert C. Van Loon and Corné M.J. Pieterse
Section of Phytopathology, Faculty of Biology, Utrecht University, Graduate School Experimental Plant Sciences, P.O. Box 800.84, 3508 TB Utrecht, The Netherlands (Phone: +31302537438; Fax: +31302518366; E-mail: J.Ton@bio.uu.nl; Internet: http://www.bio.uu.nl/~fytopath)

Accepted 17 September 2000

Key words: biological control, ethylene, jasmonic acid, *Pseudomonas fluorescens*, SA-independent

Abstract

Selected strains of non-pathogenic rhizobacteria have the ability to trigger an induced systemic resistance (ISR) response in plants. In Arabidopsis, rhizobacteria-mediated ISR has been extensively studied, using *Pseudomonas fluorescens* WCS417r as the inducing agent and *P. syringae* pv. *tomato* DC3000 (*Pst*) as the challenging pathogen. To investigate how far expression of ISR depends on the level of basal resistance, 10 different Arabidopsis ecotypes were screened for their potential to express WCS417r-mediated ISR and basal resistance against *Pst*. Two Arabidopsis ecotypes, RLD and Wassilewskija (Ws), were found to be blocked in their ability to express ISR. This ISR-noninducible phenotype correlated with a relatively low level of basal resistance against *Pst*. Genetic analysis of crosses between the ISR-inducible ecotypes Columbia (Col) and Landsberg *erecta* (L*er*), on the one hand, and the non-inducible ecotypes RLD and Ws, on the other hand, revealed that ISR inducibility and basal resistance against *Pst* were inherited as monogenic dominant traits that are genetically linked. Neither ISR inducibility, nor basal resistance against *Pst* was complemented in the F_1 progeny of a cross between RLD and Ws, indicating that both ecotypes are affected in the same locus. This locus, designated *ISR1*, was mapped between markers *Ein3* and *GL1* on chromosome III. Interestingly, ecotypes RLD and Ws also failed to express ISR against the oomycetous pathogen *Peronospora parasitica*, but they were not affected in their level of basal resistance against this pathogen. Thus, the *ISR1* locus controls the expression of ISR against different pathogens but basal resistance only against *Pst* and not against *P. parasitica*. Like ecotypes RLD and Ws, ethylene-insensitive mutants showed the *isr1* phenotype in that they were unable to express WCS417r-mediated ISR and show enhanced susceptibility to *Pst* infection. Analysis of ethylene responsiveness of RLD and Ws revealed that both ecotypes exhibit reduced sensitivity to ethylene. Therefore, it is proposed that the Arabidopsis *ISR1* locus encodes a component of the ethylene-response pathway that plays an important role in ethylene-dependent resistance mechanisms.

Abbreviations: CAPS – cleaved amplified polymorphic sequence; ISR – induced systemic resistance; JA – jasmonic acid; PRs – pathogenesis-related proteins; *Pst* DC3000 – *Pseudomonas syringae* pv. *tomato D*C3000; SA – salicylic acid; SAR – systemic acquired resistance.

Systemically induced disease resistance

When appropriately stimulated, plants systemically enhance their defensive capacity against pathogen attack. This induced resistance is generally characteriseds by a restriction of pathogen growth and a reduction in disease severity (Hammerschmidt, 1999). The development of a broad-spectrum, systemic acquired

resistance (SAR) after primary pathogen infection has been studied extensively (Ryals et al., 1996). This pathogen-induced SAR is characterised by an early increase in endogenously synthesised salicylic acid (SA) (Malamy et al., 1990; Métraux et al., 1990). SA is an essential signalling compound in the SAR signalling pathway, because transgenic plants that are unable to accumulate SA are incapable of developing SAR (Gaffney et al., 1993). Furthermore, SAR is associated with the systemic activation of so-called SAR genes. These include genes that encode pathogenesis-related (PR) proteins (Ward et al., 1991), some of which have *in vivo* antifungal activity and are therefore thought to contribute to the resistance state of SAR (Ryals et al., 1996; Van Loon, 1997).

Selected strains of non-pathogenic, root-colonising rhizobacteria have been shown to be capable of inducing disease resistance as well. This phenomenon is commonly referred to as rhizobacteria-mediated induced systemic resistance (ISR; Van Loon et al., 1998). Rhizobacteria are present in large numbers on the root surface, where plant exudates and lysates provide nutrients (Lynch and Whipps, 1991). Besides inducing resistance, rhizobacterial strains have been reported to also directly antagonise soil-borne pathogens and to stimulate plant growth (Bakker et al., 1991; Wei et al., 1996; Pieterse and Van Loon, 1999). Since the discovery of the resistance-inducing capacities of rhizobacteria in the early 1990s, ISR has been demonstrated in different plant species against a broad spectrum of pathogens (Van Loon et al., 1998). The mechanisms by which rhizobacteria induce resistance vary. Some rhizobacterial strains trigger the SA-dependent resistance pathway by producing SA at the root surface (De Meyer and Hofte, 1997; Maurhofer et al., 1998; De Meyer et al., 1999), whereas others activate a SA-independent pathway (Pieterse et al., 1996; Press et al., 1997).

Arabidopsis thaliana has proved to be an attractive model species for the elucidation of the molecular mechanisms underlying rhizobacteria-mediated ISR. Using *Pseudomonas fluorescens* WCS417r as the inducing agent, it was demonstrated that ISR occurred without concomitant expression of SAR genes (Pieterse et al., 1996; Van Wees et al., 1999). Moreover, the ISR signalling pathway functioned independently of SA, but required an intact response to the plant hormones jasmonate (JA) and ethylene (Pieterse et al., 1998; Knoester et al., 1999). Further investigations revealed that induction of ISR did not result in enhanced production of JA and ethylene (Pieterse et al., 2000), nor in the expression of ethylene- and JA-responsive genes (Van Wees et al., 1999). These observations suggest that ISR is based on a sensitisation of the tissue for these hormones, rather than on an increase in their production. Despite the differences between WCS417r-mediated ISR and pathogen-induced SAR, both resistance responses were found to be blocked in the *npr1-1* mutant, indicating that both SAR and ISR are controlled by the regulatory protein NPR1 (Cao et al., 1994; Pieterse et al., 1998). Downstream of NPR1 both pathways diverge, indicating that NPR1 differentially regulates defence responses depending on the pathway that is activated upstream of it (Pieterse et al., 1998). Interestingly, simultaneous activation of both the JA/ethylene-dependent ISR pathway and the SA-dependent SAR pathway resulted in an enhanced level of protection against *Pseudomonas syringae* pv. *tomato* DC3000 (*Pst*) (Van Wees et al., 2000), indicating that the effects of ISR and SAR are additive.

Naturally occurring variation in ISR inducibility and basal resistance against *Pst*

The capacity to express *P. fluorescens* WCS417r-mediated ISR was found to be dependent on the plant genotype. For instance, ecotypes Columbia (Col) and Landsberg *erecta* (L*er*) were responsive to induction of ISR by WCS417r, whereas ecotype RLD was not (Van Wees et al., 1997; Ton et al., 1999). In carnation, cultivar specificity with regard to expression of WCS417r-mediated ISR has also been reported. ISR induced by WCS417r against fusarium wilt, caused by *Fusarium oxysporum* f.sp. *dianthi*, was clearly expressed in the moderately resistant cultivar Pallas, but less strongly and consistently in the susceptible cultivar Lena (Van Peer et al., 1991). This suggests that the level of basal resistance can influence the extent to which WCS417r-mediated ISR is expressed. Such observations fit the hypothesis that induced resistance constitutes an enhancement of extant defensive mechanisms (Van Loon, 1997). However, several situations have been reported in which a clear correlation between the capacity to express rhizobacteria-mediated ISR and the level of basal resistance against the challenging pathogen was absent. For instance, in cucumber two susceptible cultivars expressed ISR after treatment with *Serratia marcescens* 90-166, whereas a resistant cultivar did not (Liu et al., 1995). Moreover, both susceptible and resistant cultivars of radish were capable

of expressing *P. fluorescens* WCS374-mediated ISR against fusarium wilt to the same extent (Leeman et al., 1995).

To further elucidate the relationship between *P. fluorescens* WCS417r-mediated ISR and basal resistance against *Pst* in Arabidopsis, a genetic approach using the natural variation in ISR inducibility between ecotypes was persued. Out of the 10 ecotypes tested, RLD and Wassilewskija (Ws) failed to develop ISR after treatment of roots with WCS417r bacteria, whereas they were fully capable of expressing SAR after predisposal infection with avirulent *Pst*(*avrRpt2*) (Table 1; Ton et al., 1999). This ISR-noninducible phenotype of RLD and Ws could not be attributed to poor root colonisation by the ISR-inducing WCS417r bacteria, because both ecotypes allowed levels of root colonisation comparable to that of the ISR-inducible ecotypes (Ton et al., 1999). Remarkably, the ISR-noninducible phenotype of RLD and Ws correlated with a relatively low level of basal resistance against *Pst*. After challenge inoculation, disease symptoms in RLD and Ws were characterised by many large necrotic or water-soaked lesions, surrounded by extensive chlorosis, whereas disease on Col plants was more restricted and significantly less severe (Ton et al., 1999). Moreover, RLD and Ws allowed at least 5-fold higher levels of growth of *Pst* compared to the ISR-inducible ecotypes (Table 1; Ton et al., 1999). Apparently, RLD and Ws lack one or more genetic traits that are not only involved in the expression of ISR, but also contribute to basal resistance against *Pst*.

Table 1. Quantification of *P. fluorescens* WCS417r-mediated ISR, pathogen-induced SAR and basal resistance against *Pst* in different Arabidopsis ecotypes[a]

Ecotype[a]	ISR (% induced protection)[a]	SAR (% induced protection)[a]	Basal resistance[b]
Col	42.2*	61.8*	2.36 ± 0.18
L*er*	45.8*	57.8*	2.83 ± 0.08
Cvi	19.3*	37.6*	3.06 ± 0.17
Sha	32.3*	43.1*	2.71 ± 0.35
Kas	17.0*	45.2*	2.17 ± 0.23
C24	25.7*	38.7*	2.12 ± 0.09
Wei	27.9*	38.0*	2.96 ± 0.22
Ren	41.5*	54.9*	2.77 ± 0.18
RLD	−4.9	62.8*	3.61 ± 0.09
Ws	−5.3	42.8*	3.71 ± 0.21

[a]ISR was induced by transferring 2-week-old seedlings to soil containing *P. fluorescens* WCS417r bacteria at 5×10^7 cfu g^{-1}. SAR was induced by pressure infiltrating a suspension of avirulent *Pst* (*Pst*(*avrRpt2*)) at 10^7 cfu ml^{-1} into two lower leaves 4 days prior to challenge inoculation. Five-week-old plants were challenge-inoculated by dipping the leaves in a bacterial suspension of *Pst* at 2.5×10^7 cfu ml^{-1}. Three to 5 days later, the plants were scored for disease symptoms. Induced protection is presented as the reduction in percentage of leaves with disease symptoms, relative to that of challenged control plants. Asterisks indicate statistically significant differences compared to non-induced control plants (Fisher's LSD test; $\alpha = 0.05$; $n = 20$–25). [b]Values presented are means (± SD) of the log of the proliferation of *Pst* over a 3-day time interval. Five-week-old plants were infected by pressure infiltrating a suspension of virulent *Pst* at 5×10^5 cfu ml^{-1} into the leaves. Immediately after pressure infiltration and 3 days later, the number of *Pst* bacteria per gram of leaf fresh weight was determined.

Identification of the *ISR1* locus controlling both WCS417r-mediated ISR and basal resistance against *Pst*

The naturally occurring variation in ISR inducibility and basal resistance against *Pst* enabled a genetic analysis to be performed by crossing the ISR-noninducible ecotypes RLD and Ws with the ISR-inducible ecotypes Col and L*er*. The resulting F_1 progenies were all capable of expressing ISR and showed comparable levels of growth of *Pst* in their leaves as the corresponding ISR-inducible parents (Table 2; Ton et al., 1999). These results demonstrated that ISR inducibility and basal resistance against *Pst* are both inherited as dominant traits. To investigate whether these traits are mono- or multigenic, the F_2 progeny of the RLD × Col cross was examined for segregation of ISR inducibility and basal resistance. Of the 98 F_2 plants tested, 28 plants were nonresponsive to WCS417r treatment and exhibited a level of disease severity comparable to that of RLD plants, whereas 70 plants were responsive to WCS417r treatment and showed disease severity similar to that in ISR-expressing Col plants. These data fit a statistically significant 3 : 1 segregation ($\chi^2 = 0.667$; $P = 0.414$) indicating that both ISR inducibility and basal resistance against *Pst* are monogenically inherited.

To further investigate the inheritance of ISR inducibility in relation to basal resistance, individual F_2 plants of the Col × RLD cross were selfed, resulting in 74 F_3 families. Subsequently, 16 non-induced F_3 plants of each family were challenge inoculated with *Pst*, whereupon the disease symptoms were monitored after 3, 4 and 5 days. Evaluation of symptoms revealed that 17 F_3 families were homozygous for the moderate

Table 2. Quantification of *P. fluorescens* WCS417r-mediated ISR and basal resistance against *Pst* in different Arabidopsis ecotypes and F_1 plants

Genotype	ISR (% induced protection)[a]	Basal resistance[b]
Col	35.7*	2.36 ± 0.18
Col × RLD	49.9*	2.24 ± 0.15
Col × Ws	50.0*	2.35 ± 0.07
L*er*	36.0*	2.83 ± 0.08
L*er* × Ws	29.0*	2.80 ± 0.08
RLD	−3.1	3.61 ± 0.09
Ws	−0.5	3.71 ± 0.21
Ws × RLD	− 0.1	3.51 ± 0.24

[a]ISR was induced by transferring 2-week-old seedlings to soil containing *P. fluorescens* WCS417r bacteria at 5×10^7 cfu g^{-1}. Five-week-old plants were challenge-inoculated by dipping the leaves in a bacterial suspension of *Pst* at 2.5×10^7 cfu ml^{-1}. Three days after challenge inoculation, the plants were scored for disease symptoms. Induced protection is presented as the reduction in percentage of leaves with disease symptoms, relative to that of challenged control plants. Asterisks indicate statistically significant differences compared to non-induced control plants (Student's *t* test $\alpha = 0.05$).

[b]Values presented are means (± SD) of the log of the proliferation of *Pst* over a 3-day time interval. Five-week-old plants were infected by pressure infiltrating a suspension of virulent *Pst* at 5×10^5 cfu ml^{-1} into the leaves. Immediately after pressure infiltration and 3 days later, the number of *Pst* bacteria per gram of leaf fresh weight was determined.

Col-like disease symptoms, 40 F_3 families were heterozygous, showing both moderate and severe disease symptoms, and 17 F_3 families were homozygous for severe RLD-like disease symptoms. This segregation of disease severity in the F_3 families fits a Mendelian 1 : 2 : 1 segregation ($\chi^2 = 0.486$; $P = 0.784$), confirming the monogenic inheritance of basal resistance against *Pst*. Five RLD-like families and 5 Col-like families were tested for growth of *Pst* in the leaves and responsiveness to induction of ISR by WCS417r. The 5 F_3 families characterised by Col-like disease symptoms were fully capable of expressing WCS417r-mediated ISR and allowed relatively low levels of growth of *Pst*. In contrast, the 5 F_3 families characterised by RLD-like disease symptoms, did not develop ISR upon treatment with WCS417r and allowed relatively high levels of growth of *Pst*. This apparent co-segregation of ISR inducibility and relatively high basal resistance, on the one hand, and ISR noninducibility and relatively low basal resistance, on the other hand, demonstrates that both defence mechanisms are genetically linked. The corresponding locus was designated *ISR1* (Ton et al., 1999).

Using the homozygous RLD-like (*isr1/isr1*) and Col-like (*ISR1/ISR1*) F_3 families as genetic populations, cleaved amplified polymorphic sequence (CAPS) analysis was performed to locate the genetic map position of the *ISR1* locus. The *ISR1* locus cosegregated with the markers *B4* ($\chi^2 = 13.5$; $P = 0.001$), *Ein3* ($\chi^2 = 18.1$; $P < 0.001$), *GL1* ($\chi^2 = 18.0$; $P < 0.001$), and *BGl1* ($\chi^2 = 5.5$; $P = 0.064$) on chromosome III. Of the 32 segregants tested, 15 chromosomes were recombinant with *B4*, 13 with *Ein3*, 14 with *GL1*, and 23 with *BGL1*, yielding recombination frequencies of 23.4%, 21.9%, 20.3% and 35.9%, respectively. These recombination frequencies indicate that the *ISR1* locus is located on chromosome III between markers *Ein3* and *GL1*.

Arabidopsis ecotypes RLD and Ws are both affected in the *ISR1* locus

Ecotype Ws showed the same phenotype as RLD, and upon crossing with Col or L*er*, the phenotype was likewise found to be recessive (Table 2). To investigate whether RLD and Ws are affected in the same trait, a cross between the two ecotypes was made and the progeny was analysed for both ISR inducibility and basal resistance against *Pst*. The resulting F_1 progeny behaved as their ISR-noninducible parents: they failed to develop WCS417r-mediated ISR and allowed relatively high levels of growth of *Pst* in their leaves, comparable to that of both parents (Table 2; Ton et al., 1999). These findings demonstrated that RLD and Ws were unable to complement each other for the ability to express ISR and basal resistance against *Pst*, indicating that both ecotypes are affected in the *ISR1* locus.

The *ISR1* locus is not involved in basal resistance against *Peronospora parasitica*

The involvement of the *ISR1* locus in both ISR and basal resistance against *Pst* suggests that *ISR1* encodes a common component that is involved in both resistance responses. To investigate whether this locus similarly regulates ISR and basal resistance against a different pathogen, the resistance responses of ecotypes Col and Ws against the downy mildew-causing oomycetous leaf pathogen *P. parasitica* were studied.

Table 3. Quantification of *P. fluorescens* WCS417r-mediated ISR and basal resistance against *P. parasitica* in the Arabidopsis ecotypes Col (*ISR1/ISR1*) and Ws (*isr1/isr1*)

Ecotype	Treatment[a]	Disease rating[b] (no. leaves per class)				Total no. leaves	% leaves with spores	No. isolated sporesg^{-1} FW[c]
		0	1	2	3			
Col	Control	80	45	66	43	234	66	10.8×10^5
	WCS417r*	128	12	33	27	200	36	1.5×10^5
Ws	Control	118	21	20	21	180	34	5.3×10^5
	WCS417r	127	19	30	29	205	38	6.2×10^5

[a]ISR was induced by transferring 2-week-old seedlings to soil containing *P. fluorescens* WCS417r bacteria at 5×10^7 cfu g^{-1}. Three-week-old plants were challenge-inoculated by applying 3 µl droplets to the leaves of a spore suspension of *P. parasitica* WACO9 at 5×10^4 spores ml^{-1}. At 11 days after challenge inoculation, disease symptoms were scored and spores were isolated and counted. Asterisks indicate statistically significant different distributions of the disease-severity classes compared to the control treatments (Chi-square, $P < 0.05$).

[b]Disease rating was expressed as intensity of sporulation on each leaf: 0, no sporulation; 1, <50% of the leaf area covered by sporangia; 2, >50% of the leaf area covered by sporangia; 3, heavily covered with sporangia, with additional chlorosis and leaf collapse.

[c]FW = fresh weight.

In this plant–pathogen interaction, induced resistance was expressed both as a reduction in symptom severity and a reduction of sporulation of the pathogen. In Col (*ISR1/ISR1*), treatment of the roots with WCS417r bacteria resulted in substantial induced protection against *P. parasitica* (Table 3). In contrast, ecotype Ws (*isr1/isr1*) did not develop resistance against this pathogen after treatment of the roots with WCS417r. Thus, the *ISR1* locus is not only involved in induced resistance against the pathogenic bacterium *Pst*, but also against pathogenic oomycete *P. parasitica*. However, non-induced Ws plants infected with *P. parasitica* did not exhibit enhanced disease susceptibility compared to Col (Table 3). Apparently, the *ISR1* locus does not contribute to basal resistance against *P. parasitica*, although it is involved in the regulation of ISR against this pathogen.

Physiological characterisation of the *ISR1* phenotype

As described above, the *ISR1* locus in Arabidopsis is involved in ISR against different pathogens as well as specific basal resistance against *Pst*, whereas it does not contribute to basal resistance against the oomycetous pathogen *P. parasitica*. Interestingly, the ethylene response mutant *etr1-1* and the JA response mutant *jar1-1* exhibited a similar phenotype as ecotypes RLD and Ws, in that they were blocked in WCS417r-mediated ISR, affected in basal resistance against *Pst*, but unaffected in basal resistance against *P. parasitica* (Pieterse et al., 1998; Thomma et al., 1998; Ton et al., 1999). This resemblance in phenotypic characteristics suggests that the *ISR1* locus could be involved in the JA/ethylene-dependent signalling. Indeed, recent results indicate that RLD (*isr1/isr1*) and Ws (*isr1/isr1*) showed reduced sensitivity to ethylene in comparison to Col (*ISR1/ISR1*), as determined by both physiological responses and ethylene-dependent gene expression. This reduced sensitivity to ethylene cosegregated with the recessive alleles at the *ISR1* locus (Ton et al., 2000). Therefore, it is proposed that the *ISR1* locus encodes a component of the ethylene-response pathway that plays a role in ethylene-dependent resistance responses. Future research will be directed towards cloning the gene at the *ISR1* locus, and characterising it in relation to the role of ethylene in basal resistance against pathogens and rhizobacteria-mediated ISR.

References

Bakker PAHM, van Peer R and Schippers B (1991) Suppression of soil-borne plant pathogens by fluorescent Pseudomonads: mechanisms and prospects. In: Beemster ABR et al. (eds) Biotic Interactions and Soil-Borne Diseases (pp 217–230). Elsevier Scientific Publishers, Amsterdam

Cao H, Bowling SA, Gordon AS and Dong X (1994) Characterization of an Arabidopsis mutant that is nonresponsive to inducers of systemic acquired resistance. Plant Cell 6: 1583–1592

De Meyer G and Höfte M (1997) Salicylic acid produced by the rhizobacterium *Pseudomonas aeruginosa* 7NSK2 induces

resistance to leaf infection by *Botrytis cinerea* on bean. Phytopathology 87: 588–593

De Meyer G, Capieau K, Audenaert K, Buchala A, Métraux J-P and Höfte M (1999) Nanogram amounts of salicylic acid produced by the rhizobacterium *Pseudomonas aeruginosa* 7NSK2 activate the systemic acquired resistance pathway in bean. Mol Plant–Microbe Interact 12: 450–458

Gaffney T, Friedrich L, Vernooij B, Negrotto D, Nye G, Uknes S, Ward E, Kessmann H and Ryals J (1993) Requirement of salicylic acid for the induction of systemic acquired resistance. Science 261: 754–756

Hammerschmidt R (1999) Induced disease resistance: how do induced plants stop pathogens? Physiol Mol Plant Pathol 55: 77–84

Knoester M, Pieterse CMJ, Bol JF and Van Loon LC (1999) Systemic resistance in Arabidopsis induced by rhizobacteria requires ethylene-dependent signaling at the site of application. Mol Plant–Microbe Interact 12: 720–727

Leeman M, Van Pelt JA, Den Ouden FM, Heinsbroek, Bakker M and Schippers B (1995) Biocontrol of fusarium wilt of radish by lipopolysaccharides of *Pseudomonas fluorescens*. Phytopathology 85: 1021–1027

Liu L, Kloepper JW and Tuzun S (1995) Induction of systemic resistance in cucumber by plant growth-promoting rhizobacteria: duration of protection and effect of protection and root colonization. Phytopathology 85: 1064–1068

Lynch JM and Whipps JM (1991) Substrate flow in the rhizosphere. In: Keister DL and Cregan PB (eds) The Rhizosphere and Plant Growth (pp 15–24). Kluwer, Dordrecht

Malamy J, Carr JP, Klessig DF and Raskin I (1990) Salicylic acid: a likely endogenous signal in the resistance response of tobacco to viral infection. Science 250: 1002–1004

Maurhofer M, Reimmann C, Schmidli-Sacherer P, Heeb S, Haas D and Défago G (1998) Salicylic acid biosynthetic genes expressed in *Pseudomonas fluorescens* strain P3 improve the induction of systemic resistance in tobacco against tobacco necrosis virus. Phytopathology 88: 678–684

Métraux J-P, Signer H, Ryals J, Ward E, Wyss-Benz M, Gaudin J, Raschdorf K, Schmid E, Blum W and Inverardi B (1990) Increase in salicylic acid at the onset of systemic acquired resistance in cucumber. Science 250: 1004–1006

Pieterse CMJ and Van Loon LC (1999) Salicylic acid-independent plant defence pathways. Trends Plant Sci 4: 52–58

Pieterse CMJ, Van Pelt JA, Ton J, Parchmann S, Mueller MJ, Buchala AJ, Métraux J-P and Van Loon LC (2000) Rhizobacteria-mediated induced systemic resistance (ISR) in *Arabidopsis* requires sensitivity to jasmonate and ethylene but is not accompanied by an increase in their production. Physiol Mol Plant Pathol 57: 123–134

Pieterse CMJ, Van Wees SCM, Hoffland E, Van Pelt JA and Van Loon LC (1996) Systemic resistance in *Arabidopsis* induced by biocontrol bacteria is independent of salicylic acid and pathogenesis-related gene expression. Plant Cell 8: 1225–1237

Pieterse CMJ, Van Wees SCM, Van Pelt JA, Knoester M, Laan R, Gerrits H, Weisbeek PJ and Van Loon LC (1998) A novel signaling pathway controlling induced systemic resistance in *Arabidopsis*. Plant Cell 10: 1571–1580

Press CM, Wilson M, Tuzun S and Kloepper JW (1997) Salicylic acid produced by *Serratia marcescens* 90-166 is not the primary determinant of induced systemic resistance in cucumber or tobacco. Mol Plant–Microbe Interact 10: 761–768

Ryals JA, Neuenschwander UH, Willits MG, Molina A, Steiner H-Y and Hunt MD (1996) Systemic acquired resistance. Plant Cell 8: 1809–1819

Thomma BPHJ, Eggermont K, Penninckx IAMA, Mauch-Mani B, Cammue BPA and Broekaert WF (1998) Separate jasmonate-dependent and salicylic acid-dependent defence response pathways in *Arabidopsis* are essential for resistance to distinct microbial pathogens. Proc Natl Acad Sci USA 95: 15107–15111

Ton J, Davidon, S, Van Wees SCM, Van Loon LC and Pieterse CMJ (2000) The Arabidopsis *ISR1* locus controlling rhizobacteria-mediated induced systemic resistance is involved in ethylene signaling. Plant Physiol (in press)

Ton J, Pieterse CMJ and Van Loon LC (1999) Identification of a locus in Arabidopsis controlling both the expression of rhizobacteria-mediated induced systemic resistance (ISR) and basal resistance against *Pseudomonas syringae* pv.*tomato*. Mol Plant–Microbe Interact 12: 911–918

Van Loon LC (1997) Induced resistance in plants and the role of pathogenesis-related proteins. Eur J Plant Pathol 103: 753–765

Van Loon LC, Bakker PAHM and Pieterse CMJ (1998) Systemic resistance induced by rhizosphere bacteria. Annu Rev Phytopathol 36: 453–483

Van Peer R, Niemann GJ and Schippers B (1991) Induced resistance and phytoalexin accumulation in biological control of fusarium wilt of carnation by *Pseudomonas* sp. strain WCS417r. Phytopathology 81: 728–734

Van Wees SCM, De Swart EAM, Van Pelt JA, Van Loon LC and Pieterse CMJ (2000) Enhancement of induced disease resistance by simulataneous activation of salicylate- and jasmonate-dependent defense pathways in *Arabidopsis thalinana*. Proc Natl Acad Sci USA 97: 8711–8716

Van Wees SCM, Luijendijk M, Smoorenburg I, Van Loon LC and Pieterse CMJ (1999) Rhizobacteria-mediated induced systemic resistance (ISR) in *Arabidopsis* is not associated with a direct effect on known defense-related genes but stimulates the expression of the jasmonate-inducible gene *Atvsp* upon challenge. Plant Mol Biol 41: 537–549

Van Wees SCM, Pieterse CMJ, Trijssenaar A, Van 't Westende YAM, Hartog F and Van Loon LC (1997) Differential induction of systemic resistance in Arabidopsis by biocontrol bacteria. Mol Plant–Microbe Interact 6: 716–724

Ward ER, Uknes SJ, Williams SC, Dincher SS, Wiederhold DL, Alexander DC, Ahl-Goy P, Métraux J-P and Ryals JA (1991) Co-ordinate gene activity in response to agents that induce systemic acquired resistance. Plant Cell 3: 1085–1094

Wei G, Kloepper JW and Tuzun S (1996) Induced systemic resistance to cucumber diseases and increased plant growth by plant growth-promoting rhizobacteria under field conditions. Phytopathology 86: 221–224