Signalling in Rhizobacteria-Induced Systemic Resistance in *Arabidopsis thaliana*

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Abstract: **To protect themselves from disease, plants have evolved sophisticated defence mechanisms in which the signal molecules salicylic acid, jasmonic acid and ethylene often play crucial roles. Elucidation of signalling pathways controlling disease resistance is a major objective in research on plant–pathogen interactions. The capacity of a plant to develop a broad spectrum, systemic acquired resistance (SAR) after primary infection with a necrotizing pathogen is well-known and its signal transduction pathway extensively studied. Plants of which the roots have been colonized by specific strains of non-pathogenic fluorescent *Pseudomonas* spp. develop a phenotypically similar form of protection that is called rhizobacteria-mediated induced systemic resistance (ISR). In contrast to pathogen-induced SAR, which is regulated by salicylic acid, rhizobacteria-mediated ISR is controlled by a signalling pathway in which jasmonic acid and ethylene play key roles. In the past eight years, the model plant species *Arabidopsis thaliana* was explored to study the molecular basis of rhizobacteria-mediated ISR. Here we review current knowledge of the signal transduction steps involved in the ISR pathway that leads from recognition of the rhizobacteria in the roots to systemic expression of broad-spectrum disease resistance in aboveground foliar tissues.**

Key words: *Arabidopsis*, **disease resistance, ethylene, induced systemic resistance, jasmonic acid, plant defence, *Pseudomonas fluorescens*, systemic acquired resistance.**

Introduction

Rhizobacteria are present on the root surface, where plant exudates and lysates provide nutrients (Lynch and Whipps, 1991[41]). Certain strains of rhizobacteria are referred to as plant growth-promoting rhizobacteria (PGPR), because they can stimulate growth of plants (Kloepper et al., 1980[31]). Growth promotion results mainly from suppressing soil-borne pathogens and other deleterious micro-organisms (Schippers et al., 1987[61]), but direct effects on plant growth have also been reported (Lynch, 1976[40]; Van Peer and Schippers, 1989[78]). Fluorescent *Pseudomonas* spp. are among the most effective PGPR and have been shown to be responsible for the reduction of soil-borne diseases in natural disease-suppressive soils (Raaijmakers and Weller, 1998[56]). The biological control activity of selected *Pseudomonas* spp. strains is effective under field conditions (Tuzun and Kloepper, 1995[73]; Wei et al., 1996[88]) and in commercial greenhouses (Leeman et al., 1995 c[39]), and can be the result of competition for nutrients, siderophore-mediated competition for iron, or antibiosis (Bakker et al., 1991[1]).

In 1991, two research groups independently demonstrated that besides a direct antagonistic effect on soil-borne pathogens, fluorescent *Pseudomonas* spp. can have an indirect effect on different types of pathogens as well. Application of selected *Pseudomonas* spp. strains were shown to be capable of triggering a plant-mediated resistance response in aboveground plant parts (Van Peer et al., 1991[79]; Wei et al., 1991[87]). This type of induced resistance is often referred to as rhizobacteria-mediated induced systemic resistance (ISR), and has been demonstrated in many plant species, e.g., bean, carnation, cucumber, radish, tobacco, tomato and the model plant *Arabidopsis thaliana* (Van Loon et al., 1998[76]). Phenotypically, rhizobacteria-mediated ISR resembles classic pathogen-induced resistance, in which non-infected parts of previously pathogen-infected plants become more resistant to further infection. This latter form of induced resistance is often referred to as systemic acquired resistance (SAR) (Ross, 1961[58]). Although the terms SAR and ISR are synonymous (Hammerschmidt et al., 2001[27]), for convenience we distinguish between pathogen- and rhizobacteria-induced resistance by using the term SAR for the pathogen-induced type and ISR for the rhizobacteria-induced type of resistance. Here we will review the current knowledge of rhizobacteria-mediated ISR and show that plants possess an ingenious network of defence-signalling pathways that provides protection against attack by different types of pathogens.

Arabidopsis as a Model to Study Rhizobacteria-Mediated ISR

In the past decade, the molecular mechanism of pathogen-induced SAR has been studied extensively, and several excellent reviews provide insight in the progress made in this fast developing field of research (Delaney, 1997[15]; Dempsey et al., 1999[16]; Dong, 2001[19]; Hammerschmidt, 1999[26]; Métraux, 2001[47]; Ryals et al., 1996[60]; Sticher et al., 1997[63]). Our un-

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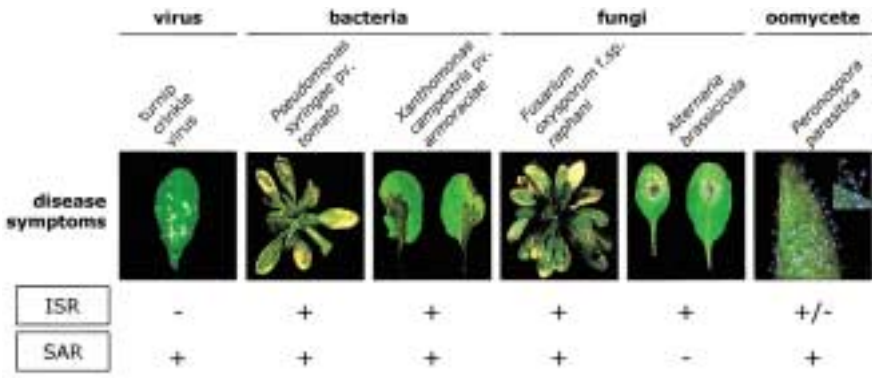

Fig. 1 Spectrum of effectiveness of rhizobacteria-mediated ISR and pathogen-induced SAR in *Arabidopsis*. Photographs show non-induced plants with symptoms caused by the respective pathogens. The effectiveness of ISR and SAR against these pathogens is indicated with + and – signs and was assessed as described previously (Pieterse et al., 1996[49]; Ton et al., 2002 b[71]; Van Wees et al., 1997[81]).

derstanding of the molecular mechanisms involved in rhizobacteria-mediated ISR also progressed. To study rhizobacteria-mediated ISR, an *Arabidopsis*-based model system was developed because this plant species was proven to be excellently suited for molecular genetic research on plant–microbe interactions (Kunkel, 1996[34]; Mauch-Mani and Slusarenko, 1993[43]). In this model system, the non-pathogenic rhizobacterial strain *Pseudomonas fluorescens* WCS417r is used as the inducing agent (Pieterse et al., 1996[49]), as this strain has been shown to trigger ISR in several plant species, e.g., carnation (Van Peer et al., 1991[79]), radish (Leeman et al., 1995 a[37]), tomato (Duijff et al., 1998[20]) and bean (Bigirimana and Höfte, 2002[4]). Colonization of *Arabidopsis* roots by ISR-inducing *P. fluorescens* WCS417r bacteria protects the plants against different types of pathogens, including the bacterial leaf pathogens *Pseudomonas syringae* pv. *tomato* and *Xanthomonas campestris* pv. *armoraciae,* the fungal root pathogen *Fusarium oxysporum* f.sp. *raphani,* the fungal leaf pathogen *Alternaria brassicicola* and the oomycete leaf pathogen *Peronospora parasitica* (Pieterse et al., 1996[49]; Ton et al., 2002 b[71]; Van Wees et al., 1997[81]). Protection against these pathogens is typically manifested as both a reduction in disease symptoms and inhibition of pathogen growth. Since the rhizobacteria remain localized on the roots and thereby spatially separated from the challenging pathogen, it was concluded that the mode of action of disease suppression is through the activation of ISR in the plant.

Activation of Rhizobacteria-Mediated ISR

The ability to develop ISR in response to selected strains of rhizosphere bacteria has been documented for many different plant species (Van Loon et al., 1998[76]) and appears to depend on the host/rhizobacterium combination. For instance, *Pseudomonas putida* WCS358r and *P. fluorescens* WCS374r perform differently on different plant species: *Arabidopsis* is responsive to *P. putida* WCS358r, whereas radish and carnation are not (Leeman et al., 1995 a[37]; Van Peer et al., 1991[79]; Van Peer and Schippers, 1992[80]; Van Wees et al., 1997[81]). Conversely, radish is responsive to *P. fluorescens* WCS374r, whereas *Arabidopsis* is not (Leeman et al., 1995 a[37]; Van Wees et al., 1997[81]). This suggests that specific recognition between the plant and the ISR-inducing rhizobacterium is required for the induction of ISR. Research on the rhizobacterial determinants involved in the elicitation of ISR revealed several bacterial traits as potential inducers of ISR, including outermembrane lipopolysaccharides and iron-regulated siderophores (Leeman et al., 1995 b[38]; Van Loon et al., 1998[76]; Van Peer and Schippers, 1992[80]).

Differential Effectiveness of ISR and SAR

One of the parallels between rhizobacteria-mediated ISR and pathogen-induced SAR is that both types of induced resistance are effective against a broad spectrum of plant pathogens (Kuc, 1982[33]; Van Loon et al., 1998[76]). To compare the spectrum of effectiveness of ISR and SAR, a range of viral, bacterial, fungal and oomycete pathogens of *Arabidopsis* were tested. Both *P. fluorescens* WCS417r-mediated ISR and SAR induced by an avirulent strain of the pathogen *P. syringae* pv. *tomato* appeared to be effective against bacterial speck and black rot disease caused by the bacterial pathogens *P. syringae* pv. *tomato* and *X. campestris* pv. *armoraciae,* respectively (Fig. **1**) (Pieterse et al., 1996[49]; Ton et al., 2002 b[71]). Also fusarium wilt disease caused by the fungus *F. oxysporum* f.sp. *raphani* was equally affected by defence responses expressed during ISR and SAR (Pieterse et al., 1996[49]; Van Wees et al., 1997[81]). Moreover, disease caused by the downy mildew pathogen *P. parasitica* was inhibited in both cases, although SAR was significantly more effective than ISR (Ton et al., 2002 b[71]). Besides these similarities in effectiveness, there are also clear differences. For instance, ISR-expressing plants show enhanced resistance against infection by the fungus *A. brassicicola,* whereas SAR is not effective against this pathogen. Conversely, expression of SAR inhibits multiplication of turnip crinkle virus and strongly reduces disease symptoms caused by this virus, whereas ISR has no effect at all (Ton et al., 2002 b[71]). Thus, the spectrum of effectiveness of ISR and SAR partly overlaps but is clearly also divergent, suggesting that the defence responses activated during both types of induced resistance are, at least partly, dissimilar.

ISR and SAR are Regulated by Distinct Signalling Pathways

SAR signal transduction pathway

Early research on molecular mechanisms involved in induced disease resistance was mainly focussed on pathogen-induced SAR in tobacco, cucumber and bean plants. It was demonstrated that the onset of SAR is accompanied by a local and systemic increase in the endogenous levels of SA (Malamy et al.,

1990[42]; Métraux et al., 1990[46]) and the concomitant up-regulation of a large set of genes (Ward et al., 1991[86]), including ones encoding pathogenesis-related (PR) proteins (Van Loon and Van Strien, 1999[77]). Several PR proteins possess antimicrobial activity and are thought to contribute to the state of resistance attained. Exogenous application of SA, or functional SA analogues, such as 2,6-dichloroisonicotinic acid (INA) or benzothiadiazole (BTH), induces SAR and activates *PR* genes (Ryals et al., 1996[60]). Conversely, transgenic NahG plants expressing the bacterial salicylate hydroxylase gene *nahG,* are unable to accumulate SA and are compromised in SAR (Gaffney et al., 1993[22]), demonstrating that SA is both necessary and sufficient for induction of SAR.

To further dissect the SAR signal transduction pathway, *Arabidopsis* emerged as an excellent model species because it displayed the same SAR characteristics as observed in other plant species (Cameron et al., 1994[7]; Lawton et al., 1995[36]; Mauch-Mani and Slusarenko, 1994[44]; Uknes et al., 1992[74]). Genetic screens for SAR compromised *Arabidopsis* mutants revealed a series of mutants that all appeared to be affected in the same gene (Cao et al., 1994[8]; Delaney et al., 1995[14]; Glazebrook et al., 1996[23]; Shah et al., 1997[62]). This gene was designated *npr1* (for non-expresser of *PR* genes), or *nim1* (for non-inducible immunity). Mutant *npr1* plants accumulate normal levels of SA after pathogen infection but are impaired in their ability to express *PR* genes and to mount an SAR response, indicating that NPR1 functions downstream of SA in the SAR pathway. The *NPR1* gene encodes a protein with ankyrin-like repeats (Cao et al., 1997[9]; Ryals et al., 1997[59]), which are known to mediate protein–protein interactions and are present in proteins with diverse functions (Bork, 1993[5]). Recently, evidence was provided demonstrating that, upon induction of SAR, NPR1 is translocated to the nucleus (Kinkema et al., 2000[30]), where it activates *PR* gene expression by physically interacting with a subclass of basic leucine zipper protein transcription factors that bind to promoter sequences required for SA-inducible *PR* gene expression, as demonstrated both *in vitro* (Després et al., 2000[17]; Zhang et al., 1999[89]; Zhou et al., 2000[90]) and *in vivo* (Fan and Dong, 2002[21]; Subramaniam et al., 2001[64]). In Fig. **2** the main characteristics of the SAR signalling pathway are depicted.

ISR signal transduction pathway

Research on the molecular mechanism of rhizobacteria-mediated ISR was initially focussed on the role of PR proteins, as the accumulation of these proteins was considered to be strictly correlated with induced disease resistance. However, radish plants of which the roots were treated with ISR-inducing *P. fluorescens* WCS417r did not accumulate PR proteins, although these plants clearly showed enhanced resistance against fusarium wilt disease (Hoffland et al., 1995[28]). Similarly, *Arabidopsis* plants expressing *P. fluorescens* WCS417r-mediated ISR showed enhanced resistance against *F. oxysporum* f.sp. *raphani* and *P. syringae* pv. *tomato,* but this did not coincide with the activation of the SAR marker genes *PR-1, PR-2* and *PR-5* (Pieterse et al., 1996[49]; Van Wees et al., 1997[81]). After refuting the dogma that systemically induced disease resistance strictly coincides with accumulation of PR proteins, we decided to investigate the ISR signalling pathway in more detail. To dissect the ISR signalling pathway, the availability of the wealth of well characterized *Arabidopsis* mutants and transgenics,

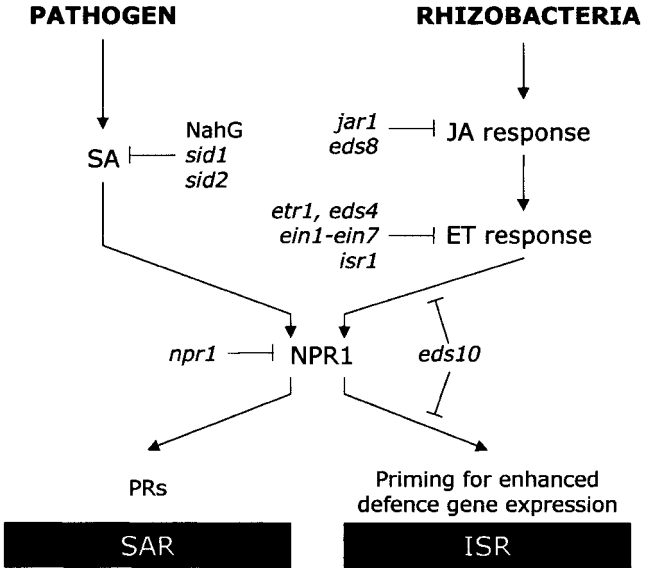

Fig. 2 Schematic model describing the pathogen-induced SAR and the rhizobacteria-mediated ISR signal transduction pathways in *Arabidopsis* (see text for details).

generated by the *Arabidopsis* community, was highly instrumental. Table **1** shows the list of *Arabidopsis* genotypes tested to date and provides references to experimental details.

The role of SA in ISR was studied in SA non-accumulating *Arabidopsis* NahG plants. In contrast to pathogen-induced SAR, *P. fluorescens* WCS417r-mediated ISR against *P. syringae* pv. *tomato* was normally expressed in these plants (Pieterse et al., 1996[49]; Van Wees et al., 1997[81]). Likewise, the SA induction-deficient mutants *sid1-1* and *sid2-1* (Nawrath and Métraux, 1999[48]) expressed normal levels of ISR (J. A. Van Pelt and C. M. J. Pieterse, unpublished results). Moreover, determination of SA levels in ISR-expressing *Arabidopsis* plants revealed that, in contrast to SAR, ISR is not associated with increased accumulation of SA (Pieterse et al., 2000[52]). This led to the conclusion that *P. fluorescens* WCS417r-mediated ISR is an SA-independent resistance response, and that ISR and SAR are regulated by distinct signalling pathways. Apart from *P. fluorescens* WCS417r, *P. putida* WCS358r has also been demonstrated to induce the SA-independent ISR pathway in *Arabidopsis* (Van Wees et al., 1997[81]). In addition, the biological control strain *Serratia marcescens* 90-166 has been shown to induce protection in both wild-type and transgenic NahG tobacco plants against *Pseudomonas syringae* pv. *tabaci* (Press et al., 1997[55]), indicating that the ability to trigger an SA-independent pathway controlling systemic resistance is not uncommon among ISR-inducing rhizobacteria. However, not all resistance-inducing rhizobacteria trigger an SA-independent resistance. For instance, an SA-overproducing mutant of *Pseudomonas aeruginosa* 7NSK2 and a genetically modified, SA-overproducing *P. fluorescens* P3 strain have been shown to trigger the SA-dependent SAR pathway by producing SA at the root surface (De Meyer and Höfte, 1997[12]; Maurhofer et al., 1998[45]).

Table 1 Capacity of different *Arabidopsis* genotypes to express *P. fluorescens* WCS417r-mediated ISR and/or pathogen-induced SAR against *P. syringae* pv. *tomato* DC3000[1]

Genotype	Phenotype	ISR	SAR	Reference[6]
Mutants/transgenics[2]				
SA related:				
NahG	SA deficient	+	–	A, B, C, F, G
sid1-1	impaired in pathogen-induced SA production	+	–	u.r.
sid2-1	idem	+	–	u.r.
npr1-1	SA insensitive; non-expressor of *PR* genes	–	–	C, F, G, H
cpr1-1	SA overproducer; constitutive expressor of *PR* genes	++[3]	+[3]	F
JA related:				
jar1-1	affected in JA response	–	+	C, F, G, H
S-12	*LOX2* co-suppressor; no induced JA levels	+	+	G
ET related:				
etr1-1	ET insensitive	–	+	C, E, F, G, H
ein2-1	idem	–	+	E
ein3-1	idem	–	+	E
ein4-1	idem	–	+	E
ein5-1	idem	–	+	E
ein6-1	idem	–	+	E
ein7-1	idem	–	+	E
eir1-1	ET insensitive in the roots only	–/+[4]	+	E
eds mutants:				
eds3-1	enhanced disease susceptibility to *P. syringae*	+	+	H
eds4-1	idem; affected in ET response[5]	–	+	H
eds5-1	idem; allelic to *sid1*	+	–	H
eds6-1	idem	+	+	H
eds7-1	idem	+	+	H
eds8-1	idem; affected in JA response	–	+	H
eds9-1	idem	+	+	H
eds10-1	idem	–	+	H
eds11-1	idem	+	+	H
eds12-1	idem; affected in SA response	+	–	H
eds13-1	idem	+	+	H
Accessions				
Columbia	wild type	+	+	A–I
Landsberg *erecta*	idem	+	+	A, B, D
RLD	idem	–	+	B, D, I
Wassilewskija	idem	–	+	D
Weiningen	idem	+	+	D
C24	idem	+	+	D
Cape Verd. islands	idem	+	+	D
Shahdara	idem	+	+	D
Kashmir	idem	+	+	D
Renkum	idem	+	+	D
Dijon	idem	+	+	u.r.

[1] The rhizobacteria-mediated ISR and pathogen-induced SAR bioassays were performed essentially as described previously (Pieterse et al., 1996[49]). ISR was induced by growing 2-week-old plants for 3 weeks in soil containing non-pathogenic *P. fluorescens* WCS417r bacteria before they were challenge inoculated with virulent *P. syringae* pv. *tomato* strain DC3000. Pathogen-induced SAR was triggered in 5-week-old plants by pressure-infiltrating three leaves per plant with *P. syringae* pv. *tomato* DC3000 carrying the avirulence gene *avrRpt2* 3 days before challenge inoculation with virulent *P. syringae* pv. *tomato* DC3000. Four days after challenge inoculation, plants were scored for bacterial speck disease symptoms. Genotypes were considered to express ISR or SAR when the mean ($n = 20$) of the proportion of diseased leaves per plant in the induction treatment was significantly lower (Fisher's LSD test; $\alpha = 0.05$) than that of the control treatment. This table is an updated version of the one published previously (Pieterse et al., 2001 b[54]).

[2] All mutants/transgenics are in the Columbia background, except for mutant *ein6* which is in the Landsberg *erecta* background. The *eds* mutants were previously selected by direct screening for enhanced disease susceptibility against *P. syringae* pv. *maculicola* (Glazebrook et al., 1996[23]; Rogers and Ausubel, 1997[57]).

[3] Mutant *cpr1* expresses SAR constitutively (Bowling et al., 1994[6]), but shows an enhanced level of resistance after induction of ISR (indicated as ++).

[4] ISR is systemically expressed only after infiltration of 3 leaves per plant with WCS417r, not after application of WCS417r to the roots.

[5] In contrast to our results, mutant *eds4-1* was previously reported to be affected in its ability to develop SAR in response to infection by avirulent *P. syringae* pv. *maculicola* carrying *avrRpt2* (Gupta et al., 2000[25]). An explanation for this inconsistency is given in Ton et al. (2002 a[70]).

[6] A, Pieterse et al. (1996[49]); B, Van Wees et al. (1997[81]); C, Pieterse et al. (1998[50]); D, Ton et al. (1999[67]); E, Knoester et al. (1999[32]); F, Van Wees et al. (2000[84]); G, Pieterse et al. (2000[52]); H, Ton et al. (2002 a[70]); I, Ton et al. (2002 c[72]); u.r., unpublished results. The origin of the genotypes is described in the respective publications.

Genetic Dissection of the ISR Signalling Pathway

ISR requires jasmonic acid and ethylene signalling

Besides SA, the plant growth regulators jasmonic acid (JA) and ethylene (ET) have repeatedly been implicated in the regulation of primary resistance responses in plants (Pieterse and Van Loon, 1999[51]; Pieterse et al., 2001 a[53]). In many cases, infection by microbial pathogens and attack by herbivorous insects is associated with enhanced production of these hormones and a concomitant activation of distinct sets of defence-related genes. Moreover, exogenous application of these compounds often results in an enhanced level of resistance. To investigate the role of JA and ET in rhizobacteria-mediated ISR, the *Arabidopsis* JA response mutant *jar1-1* and the ET response mutant *etr1-1* were tested for their ability to express ISR. Both mutants were unable to mount resistance against *P. syringae* pv. *tomato* after colonization of the roots by *P. fluorescens* WCS417r (Pieterse et al., 1998[50]), indicating that ISR requires responsiveness to both JA and ET. In addition to *etr1-1,* a set of other well characterized *Arabidopsis* mutants that are affected at different steps of the ET signalling pathway were tested for their ability to express ISR. None of the mutants developed ISR against *P. syringae* pv. *tomato* (Knoester et al., 1999[32]), indicating that an intact ET signalling pathway is required for the expression of ISR.

To elucidate the sequence of the signalling events, the resistance-inducing ability of methyl jasmonate (MeJA) and 1-aminocyclopropane-1-carboxylate (ACC), the natural precursor of ET, was tested in wild-type, NahG, *jar1-1* and *etr1-1* plants. Like *P. fluorescens* WCS417r, MeJA and ACC were effective in inducing resistance against *P. syringae* pv. *tomato* in SA non-accumulating NahG plants, suggesting that both inducers activate the SA-independent ISR pathway. Moreover, MeJA-induced protection was blocked in both *jar1-1* and *etr1-1,* whereas ACC-induced protection was affected in *etr1-1,* but not in *jar1-1* plants. Hence, it was postulated that *P. fluorescens* WCS417r-mediated ISR follows a signalling pathway in which components from the JA and ET response are successively engaged (Pieterse et al., 1998[50]).

ISR is dependent on NPR1

NPR1 has been shown to be an important regulatory factor in the SA-dependent SAR response (Cao et al., 1994[8]). To investigate whether NPR1 is also involved in the SA-independent ISR response, *Arabidopsis* mutant *npr1* was tested for the induction of ISR. Surprisingly, mutant *npr1* plants were blocked in their ability to express *P. fluorescens* WCS417r-mediated ISR, indicating that, like pathogen-induced SAR, rhizobacteria-mediated ISR is an NPR1-dependent defence response (Pieterse et al., 1998[50]). Elucidation of the sequence of ISR signalling events revealed that NPR1 functions downstream of JA and ET in the ISR signalling pathway. Evidently, NPR1 is not only required for the SA-dependent expression of *PR* genes that are activated during SAR, but also for the JA- and ET-dependent activation of defence responses resulting from rhizobacteria-mediated ISR. This demonstrates that NPR1 is able to differentially regulate defence gene expression, depending on the signalling pathway that is activated upstream of it. One of the major challenges in our research on induced resistance signalling is to identify signalling components from the ISR and the SAR pathway that confer this specificity in NPR1-dependent defence gene activation. In Fig. **2** the main characteristics of the ISR signalling pathway are depicted.

The Role of Jasmonic Acid and Ethylene in ISR

Levels of jasmonic acid and ethylene in ISR-expressing plants

In *Arabidopsis,* both JA and ET activate specific sets of defence-related genes and, when applied exogenously, they confer resistance against *P. syringae* pv. *tomato* (Pieterse et al., 1998[50]; Van Wees et al., 1999[83]). To investigate whether ISR is associated with changes in JA/ET-responsive gene expression, Van Wees et al. (1999[83]) monitored the expression of a set of well characterized JA- and/or ET-responsive genes (i.e., *LOX1, LOX2, VSP, PDF1.2, HEL, CHI-B* and *PAL1*) in *Arabidopsis* plants expressing *P. fluorescens* WCS417r-mediated ISR. None of the genes tested were up-regulated in induced plants, neither locally in the roots, nor systemically in the leaves. This suggests that the resistance attained was not associated with major changes in the levels of either JA or ET. Indeed, analysis of local and systemic levels of JA and ET revealed that *P. fluorescens* WCS417r-mediated ISR is not associated with changes in the production of these signal molecules (Pieterse et al., 2000[52]). By using the *LOX2* co-suppressed transgenic line S-12, it was confirmed that an increase in JA production is not required for the induction or expression of ISR. Transgenic S-12 plants, that are affected in the production of JA in response to wounding (Bell et al., 1995[2]) and pathogen infection (Pieterse et al., 2000[52]), expressed normal levels of ISR. Together, these results suggest that the JA and ET dependency of ISR is based on enhanced sensitivity to these hormones, rather than on an increase in their production.

Priming of jasmonic acid- and ethylene-dependent defence responses during ISR

If the JA and ET dependency of ISR is based on enhanced sensitivity to these signal molecules, ISR-expressing plants would be expected to react faster or more strongly to JA and ET produced after pathogen infection. This hypothesis is supported by the finding that the expression of the JA-inducible gene *VSP* of *Arabidopsis* was significantly enhanced in ISR-expressing leaves after challenge with *P. syringae* pv. *tomato* compared to inoculated control plants (Van Wees et al., 1999[83]). In the same study, several other JA-responsive genes were also tested, but these failed to show an enhancement of the pathogen-induced expression level in ISR-expressing leaves, suggesting that ISR in *Arabidopsis* is associated with priming for augmented expression of a specific set of JA-responsive genes.

The role of ET in priming is more ambiguous. Like JA, ET is not produced at higher levels in systemic tissues expressing ISR, although intact responsiveness to ET is required for full expression of ISR. However, after treatment with a saturating dose of 1 mM of the ET precursor ACC, ISR-expressing plants emitted significantly more ET than ACC-treated control plants (Pieterse et al., 2000[52]). Evidently, the capacity to convert ACC to ET is increased in ISR-expressing plants. Because, in infected tissues, ACC levels rapidly increase as a result of pathogen-induced ACC synthase activity, the enhanced ACC-converting capacity observed in ISR-expressing plants potentially primes

the plant for a faster or greater production of ET upon pathogen attack. Interestingly, exogenous application of ACC has been shown to induce resistance against *P. syringae* pv. *tomato* in *Arabidopsis* (Pieterse et al., 1998[50]; Pieterse et al., 2000[52]; Van Wees et al., 1999[83]). Therefore, a faster or greater production of ET in the initial phase of infection might contribute to enhanced resistance against this pathogen.

Priming plant cells for expression of defence-related genes, leading to a faster and/or higher level of defence gene expression after challenge inoculation, emerged as a common feature of different types of induced resistance (Conrath et al., 2002[10]). Priming can explain, on the one hand, the apparent lack of changes in gene expression in induced tissues in the absence of a challenging pathogen, while on the other hand, the plant is able to react more efficiently to an invading pathogen. The molecular basis of priming is still unclear. Possibly, the level of cellular components with important roles in defence response signalling, e.g., certain transcription factors, may increase in primed cells. The increased presence of cellular signalling components might then lead to an accelerated and enhanced response, but only when the cells are challenged by a pathogen or another stress stimulus (Conrath et al., 2002[10]).

The Relationship between ISR and Basal Resistance

Induced resistance is expressed as an enhancement of basal resistance

Apart from their role in systemically induced resistance, the defence signal molecules SA, JA and ET have repeatedly been implicated in the regulation of primary resistance responses. Compelling evidence for the role of SA, JA and ET in basal resistance comes from recent genetic analyses of *Arabidopsis* mutants and transgenics that are affected in the biosynthesis or perception of these compounds. In many cases, genotypes affected in SA, JA or ET signalling show enhanced susceptibility to pathogen or insect attack (Dong, 1998[18]; Glazebrook, 2001[24]; Pieterse et al., 2001 a[53]). SA, JA and ET are involved, to different extents, in basal resistance against specific pathogens. For instance, basal resistance in *Arabidopsis* against the oomycete *P. parasitica* and TCV seems to be controlled predominantly by an SA-dependent pathway. Only SA non-accumulating NahG plants exhibit enhanced disease susceptibility to these pathogens (Delaney et al., 1994[13]; Kachroo et al., 2000[29]), whereas mutants affected in JA or ET signalling do not (Kachroo et al., 2000[29]; Thomma et al., 1998[65]). In contrast, basal resistance against the fungal pathogens *A. brassicicola* and *B. cinerea* is reduced only in JA- and ET-insensitive mutants, but not in NahG plants (Thomma et al., 1998[65]; Thomma et al., 1999[66]). Interestingly, basal resistance against the bacterial pathogens *P. syringae* pv. *tomato* and X. *campestris* pv. *armoraciae* was found to be affected in both NahG plants and in JA- and ET-response mutants (Pieterse et al., 1998[50]; Ton et al., 2002 b[71]), suggesting that basal resistance against these pathogens is controlled by the combined action of SA, JA and ET. However, the contribution of ET-dependent defence responses to the final outcome of the resistance reaction against *P. syringae* pv. *tomato* might vary depending on the environmental conditions because in some cases a role for ET in basal resistance against this pathogen could not be demonstrated (Bent et al., 1992[3]; Kus et al., 2002[35]). Comparison of the effectiveness of SA-dependent SAR and JA/ET-dependent ISR against the different *Arabidopsis* pathogens, mentioned above, revealed that SAR is predominantly effective against pathogens that in non-induced plants are resisted through SA-dependent basal resistance mechanisms, whereas ISR is predominantly effective against pathogens that in non-induced plants are resisted through JA/ET-dependent basal resistance responses (Ton et al., 2002 b[71]). Thus, SAR seems to constitute an enhancement of SA-dependent defences, whereas ISR seems to be based on an enhancement of JA- and ET-dependent defences.

Characterization of enhanced disease susceptibility mutants

Because of the association between induced resistance and basal resistance, we made use of a collection of *Arabidopsis eds* (enhanced disease susceptibility = reduced basal resistance) to pathogenic *P. syringae* bacteria to identify putative novel players in the ISR signalling pathway. Therefore, 11 *eds* mutants were screened for their potential to express ISR against *P. syringae* pv. *tomato*. Out of 11 *eds* mutants tested, *eds4-1, eds8-1* and *eds10-1* were non-responsive to induction of ISR by *P. fluorescens* WCS417r (Ton et al., 2002 a[70]). Further analysis of the ISR-impaired *eds* mutants revealed that they are insensitive to induction of resistance by MeJA (*eds4-1, eds8-1* and *eds10-1*) or ACC (*eds4-1* and *eds10-1*) application. Moreover, *eds4-1* and *eds8-1* showed reduced expression of the JA- and ET-responsive *PDF1.2* gene after treatment with MeJA and ACC, which was associated with a reduced sensitivity to either ET *(eds4-1)* or MeJA *(eds8-1)*. Although blocked in rhizobacteria-, MeJA- and ACC-induced protection, mutant *eds10-1* showed normal responsiveness to both MeJA and ACC, suggesting that this mutant is affected downstream of JA and ET in the ISR signalling pathway. Together, these results demonstrated that EDS4, EDS8 and EDS10 are required for ISR and act in either the JA response (EDS8), the ET response (EDS4), or downstream of the JA and ET response (EDS10) in the ISR signalling pathway (Fig. **2**) (Ton et al., 2002 a[70]). Future research should reveal the exact role of these signalling components in the expression of ISR.

Identification of a novel locus (ISR1) controlling rhizobacteria-mediated ISR

In a genetic approach to identify novel components from the ISR signalling pathway, 10 *Arabidopsis* accessions were screened for their potential to express ISR against *P. syringae* pv. *tomato* (Ton et al., 1999[67]). Of the 10 accessions tested, RLD and Wassilewskija did not develop ISR after treatment of the roots with *P. fluorescens* WCS417r. As in the ISR-minus *eds* mutants, mentioned above, the *P. fluorescens* WCS417r non-responsive phenotype was associated with a relatively high susceptibility to *P. syringae* pv. *tomato,* which was apparent as both a greater proliferation of the pathogen in the leaves and the development of more severe disease symptoms. Genetic analysis of the progeny of a cross between the *P. fluorescens* WCS417r responsive accession Columbia and the *P. fluorescens* WCS417r non-responsive accession RLD, revealed that both the potential to express ISR and the relatively high level of basal resistance against *P. syringae* pv. *tomato* are monogenic, dominant traits that are genetically linked. The corresponding locus, designated *ISR1,* was mapped on chromosome III (Ton et al., 1999[67]) and was shown to be required for ISR against different pathogens (Ton et al., 2002 c[72]).

Interestingly, mutants *jar1-1* and *etr1-1,* that are affected in their response to JA and ET, respectively, showed the same phenotype as accessions RLD and Wassilewskija in that they were both unable to express *P. fluorescens* WCS417r-mediated ISR and showed enhanced susceptibility to infection by *P. syringae* pv. *tomato* (Pieterse et al., 1998[50]). Analysis of the ET responsiveness of RLD and Wassilewskija revealed that both accessions have a reduced sensitivity to ET, that co-segregates with the recessive alleles of the *ISR1* locus (Ton et al., 2001[69]). Therefore, it was proposed that the *Arabidopsis ISR1* locus encodes a novel component of the ET response pathway that plays an important role in disease resistance signalling. Currently, we are in the process of identifying the *ISR1* gene by positional cloning.

Search for ISR-Related Genes

The state of pathogen-induced SAR is characterized by the concomitant activation of a set of *PR* genes. In SAR-expressing plants, *PR* gene products accumulated systemically to levels from 0.3 to 1 % of the total mRNA and protein content (Lawton et al., 1995[36]). However, although some PRs possess antimicrobial activity, a causal relationship between accumulation of PRs and the broad-spectrum resistance characteristic of SAR has never been convincingly demonstrated (Van Loon, 1997[75]). Over the past few years, several approaches have been initiated to identify ISR-related gene expression. The expression pattern of a large set of known, well characterized defence-related genes of *Arabidopsis* was analysed upon induction of ISR by *P. fluorescens* WCS417r. Of the many defence-related genes tested in *Arabidopsis* (e.g., the SA-inducible genes *PR-1, PR-2* and *PR-5,* and the ET- and/or JA-inducible genes *HEL, CHI-B, PDF1.2, AtVSP, LOX1, LOX2* and *PAL1*), none were found to be up-regulated in plants expressing ISR (Van Wees et al., 1999[83]). Currently, we are analysing transcript profiles of over 8000 *Arabidopsis* genes using Affymetrix GeneChip *Arabidopsis* Genome Arrays. Preliminary data confirm that, unlike SAR, the onset of ISR is not associated with major changes in gene expression (Verhagen et al., 2001[85]). Nevertheless, ISR expressing plants are clearly more resistant to different types of pathogens. Therefore, plants must possess as yet undiscovered defence-related gene products that contribute to broad-spectrum disease resistance.

Combining ISR and SAR to Improve Biocontrol of Plant Diseases

Plant diseases are responsible for large crop losses in agriculture. Conventional disease control is based on application of chemical agents and resistance breeding. The use of chemical agents and their persistence in soil are potentially harmful to the environment, especially when chemicals are applied repeatedly in large amounts, such as in the control of soil-borne fungal pathogens. Classic resistance breeding depends on the availability of resistance genes, which often show limited durability. Moreover, both these disease control strategies are directed against a single or a small group of plant pathogens. Induced disease resistance is an attractive alternative form of plant protection, as it is based on the activation of extant resistance mechanisms in the plant and is effective against a broad spectrum of plant pathogens (Kuc, 1982[33]; Van Loon et al., 1998[76]). Therefore, detailed knowledge of the molecular mechanisms underlying induced disease resistance will be instrumental in developing biologically-based, environmentally-friendly, and durable crop protection.

Previously, we demonstrated that simultaneous activation of ISR and SAR results in an enhanced level of induced protection against *P. syringae* pv. *tomato* (Van Wees et al., 2000[84]). This indicates that the JA/ET-dependent ISR pathway and the SA-dependent SAR pathway act independently and additive at the level of protection against this particular pathogen. Moreover, we provided evidence that ISR and SAR confer differential protection against different types of pathogens (Ton et al., 2002 b[71]). Thus, combining both types of induced resistance can protect the plant against a complementary spectrum of pathogens, and can even result in an additive level of induced protection against pathogens that are resisted through both the JA/ET- and the SA- dependent pathways.

Biological control of plant diseases is still in its infancy, because the level of protection and its consistency are generally not sufficient to compete with conventional methods of disease control. One approach to improve the efficacy and consistency of biological control against soil-borne pathogens is to apply combinations of antagonistic micro-organisms with different mechanisms of action (De Boer et al., 1999[11]). In addition, our findings that the combination of ISR and SAR confers protection against a complementary spectrum of pathogens and results in enhanced levels of protection against specific bacterial pathogens (Van Wees et al., 2000[84]), offers great potential for integrating both forms of induced resistance in future agricultural practices.

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