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Rhizobacteria-mediated induced systemic resistance: triggering, signalling and expression

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Abstract

Selected strains of rhizosphere bacteria reduce disease by activating a resistance mechanism in the plant named rhizobacteria-mediated induced systemic resistance (ISR). Rhizobacteria-mediated ISR resembles pathogen-induced systemic acquired resistance (SAR) in that both types of induced resistance render uninfected plant parts more resistant towards a broad spectrum of plant pathogens. Some rhizobacteria trigger the salicylic acid (SA)-dependent SAR pathway by producing SA at the root surface. In other cases, rhizobacteria trigger a different signalling pathway that does not require SA. The existence of a SA-independent ISR pathway has been demonstrated in *Arabidopsis thaliana*. In contrast to pathogen-induced SAR, ISR induced by *Pseudomonas fluorescens* WCS417r is independent of SA accumulation and pathogenesis-related (*PR*) gene activation but, instead, requires responsiveness to the plant hormones jasmonic acid (JA) and ethylene. Mutant analyses showed that ISR follows a novel signalling pathway in which components from the JA and ethylene response are successively engaged to trigger a defensive state that, like SAR, is controlled by the regulatory factor NPR1. Interestingly, simultaneous activation of both the JA/ethylene-dependent ISR pathway and the SA-dependent SAR pathway results in an enhanced level of protection. Thus combining both types of induced resistance provides an attractive tool for the improvement of disease control. This review focuses on the current status of our research on triggering, signalling, and expression of rhizobacteria-mediated ISR in Arabidopsis.

Abbreviations: ACC – 1-aminocyclopropane-1-carboxylate; CAPS – cleaved amplified polymorphic sequence; GUS – β-glucuronidase; ISR – induced systemic resistance; JA – jasmonic acid; Met – methionine; PRs – pathogenesis-related proteins; *Pst* DC3000 – *Pseudomonas syringae* pv. *tomato* DC3000; SA – salicylic acid; SAM – S-adenosyl-L-methionine; SAR – systemic acquired resistance.

Introduction

Plants possess various inducible defence mechanisms to protect themselves against pathogen attack. A well-studied, classic example of biologically-induced disease resistance is activated after infection by a necrotising pathogen, rendering distant, uninfected plant parts resistant towards a broad spectrum of normally virulent pathogens (Kuć, 1982). This form of induced disease resistance is often referred to as systemic acquired resistance (SAR; Ross, 1961), and has been demonstrated in various plant–pathogen

interactions (reviewed by Hammerschmidt and Kuć, 1995; Ryals et al., 1996; Sticher et al., 1997; Hammerschmidt, 1999). The state of pathogen-induced SAR is characterised by an early increase in endogenously synthesised salicylic acid (SA; Malamy et al., 1990; Métraux et al., 1990) and the concomitant activation of genes encoding pathogenesis-related (PR) proteins (Van Loon, 1997; Ward et al., 1991). Transgenic NahG plants that express the bacterial salicylate hydroxylase (*nahG*) gene and, thus, cannot accumulate SA, are incapable of developing SAR and do not show *PR* gene activation upon pathogen infection, indicating that SA is a necessary intermediate in the SAR signalling pathway (Gaffney et al., 1993).

Rhizosphere bacteria are present in large numbers on plant root surfaces, where root exudates and lysates provide nutrients (Lynch and Whipps, 1991). Certain strains of rhizosphere bacteria stimulate plant growth and are, therefore, called plant growth-promoting rhizobacteria. Strains that were isolated from naturally disease-suppressive soils, mainly fluorescent *Pseudomonas* spp., promoted plant growth by suppressing soil-borne pathogens. This biological control activity is effective under field conditions (Tuzun and Kloepper, 1995; Wei et al., 1996) and in commercial greenhouses (Leeman et al., 1995c), and can be the result of competition for nutrients, siderophore-mediated competition for iron, or antibiosis (Bakker et al., 1991). Some of these biological control strains are also able to reduce disease through a plant-mediated mechanism that is phenotypically similar to pathogen-induced SAR, as the induced resistance is systemically activated and extends to above-ground plant parts. This type of induced disease resistance is often referred to as rhizobacteria-mediated induced systemic resistance (ISR; reviewed by Van Loon et al., 1998). Rhizobacteria-mediated ISR has been demonstrated in many plant species, e.g. bean, carnation, cucumber, radish, tobacco, tomato and the model plant *Arabidopsis thaliana*, and has been reported to be effective against a broad spectrum of plant pathogens, including fungi, bacteria and viruses (Van Loon et al., 1998).

Rhizobacteria-mediated ISR in Arabidopsis

An *Arabidopsis*-based model system has been developed to study the molecular basis underlying rhizobacteria-mediated ISR (Pieterse et al., 1996). The non-pathogenic rhizobacterial strain *Pseudomonas fluorescens* WCS417r was used as the inducing agent, because this strain had been demonstrated to trigger ISR in several plant species, e.g. carnation (Van Peer et al., 1991), radish (Leeman et al., 1995a), and tomato (Duijff et al., 1998). Colonisation of *Arabidopsis* roots by ISR-inducing *P. fluorescens* WCS417r bacteria protects the plants against different types of pathogens, including the bacterial leaf pathogens *P. syringae* pv. *tomato* DC3000 (*Pst* DC3000) and *Xanthomonas campestris* pv. *amoracia*, the fungal root pathogen *Fusarium oxysporum* f.sp. *raphani*, and the oomyceteous leaf pathogen *Peronospora parasitica* (Pieterse et al., 1996; Van Wees et al., 1997; J. Ton, unpublished results). Protection against these pathogens is typically manifested as both a reduction in disease symptoms and inhibition of pathogen growth. Since the rhizobacteria remain localised 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.

Triggering

Differential activation of rhizobacteria-mediated ISR

Elicitation of ISR depends on the host/rhizobacterium combination. For instance, *P. putida* WCS358r and *P. fluorescens* WCS374r perform differently on different plant species: *Arabidopsis* is responsive to WCS358r, whereas radish and carnation are not (Van Peer et al., 1991; Van Peer and Schippers, 1992; Leeman et al., 1995a; Van Wees et al., 1997). Conversely, radish is responsive to WCS374r, whereas Arabidopsis is not (Leeman et al., 1995a; Van Wees et al., 1997). Also differential induction of ISR occurs between Arabidopsis ecotypes. Most ecotypes, e.g. Columbia and Landsberg *erecta*, are responsive to treatment with WCS417r, whereas ecotypes RLD and Wassilewskija are not (Van Wees et al., 1997; Ton et al., 1999; Table 1). This suggests that specific recognition between the plant and the ISR-inducing rhizobacterium is required for the induction of ISR, and that rhizobacteria-mediated ISR is genetically determined.

Bacterial determinants involved in the elicitation of ISR in Arabidopsis

In radish, purified lipopolysaccharides (LPS) and LPS-containing cell wall preparations of WCS417r are as effective as live WCS417r bacteria in inducing

Table 1. Response of different Arabidopsis genotypes to induction of *P. fluorescens* WCS417r-mediated ISR and pathogen-induced SAR against *Pst* DC3000[1]

Genotype	Phenotype	ISR	SAR	Reference[5]
Ecotypes				
Columbia	Wild-type	+	+	A–G
Landsberg *erecta*	Wild-type	+	+	A, B, D
RLD	Wild-type	−	+	B, D
Wassilewskija	Wild-type	−	+	D
Weiningen	Wild-type	+	+	D
C24	Wild-type	+	+	D
Cape Verd. islands	Wild-type	+	+	D
Shahdara	Wild-type	+	+	D
Kashmir	Wild-type	+	+	D
Renkum	Wild-type	+	+	D
Dijon	Wild-type	+	+	u.r.
Mutants/transgenics[2]				
SA related				
NahG	SA deficient	+	−	A–C, F, G
sid1-1	SA deficient	+	−	u.r.
sid2-1	SA deficient	+	−	u.r.
npr1-1	SA insensitive; non-expressor of *PR* genes	−	−	C, F, G
cpr1-1	SA overproducer; constitutive expressor of *PR* genes	++[3]	+[3]	F
JA related				
jar1-1	Affected in JA response	−	+	C, F, G
S-12	*Lox2* co-suppressor; no induced JA levels	+	+	G
Ethylene related				
etr1-1	Ethylene insensitive	−	+	C, E–G
ein2-1	Ethylene insensitive	−	+	E
ein3-1	Ethylene insensitive	−	+	E
ein4-1	Ethylene insensitive	−	+	E
ein5-1	Ethylene insensitive	−	+	E
ein6-1	Ethylene insensitive	−	+	E
ein7-1	Ethylene insensitive	−	+	E
eir1-1	Ethylene insensitive in the roots only	−/+[4]	+	E

[1]The rhizobacteria-mediated ISR and pathogen-induced SAR bioassays were performed essentially as described by Pieterse et al. (1996). 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 *Pst* DC3000. Pathogen-induced SAR was triggered in 5-week-old plants by pressure-infiltrating 3 leaves per plant with *Pst* DC3000 carrying the avirulence gene *avrRpt2* 3 days before challenge inoculation with virulent *Pst* 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 statistically significantly lower (Fisher's LSD test; $\alpha = 0.05$) than that of the control treatment.
[2]All mutants/transgenics are in the Columbia background, except for mutant *ein6* which is in the Landsberg *erecta* background.
[3]Mutant *cpr1* expresses SAR constitutively, 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]A, Pieterse et al. (1996); B, Van Wees et al. (1997); C, Pieterse et al. (1998); D, Ton et al. (1999); E, Knoester et al. (1999); F, Van Wees et al. (2000); G, Pieterse et al. (2000a); u.r., unpublished results. The origin of the genotypes is described in the respective publications.

ISR (Leeman et al., 1995b). A mutant of WCS417r (WCS417rOA$^-$ or B4), which lacks the O-antigenic side chain of the LPS, had lost its ability to induce ISR (Leeman et al., 1995b), indicating that the LPS of WCS417r is a major determinant of induction of ISR in radish. Likewise, in Arabidopsis, the LPS-containing cell wall preparations of WCS417r were able to induce ISR, whereas cell wall preparations from the LPS mutant WCS417rOA$^-$ were unable to do so (Van Wees et al., 1997). However, live WCS417rOA$^-$ bacteria induced normal levels of protection against both *Pst* DC3000 and *F. oxysporum* f.sp. *raphani* (Van Wees et al., 1997), suggesting that bacterial LPS only partly accounts for the elicitation of ISR in Arabidopsis, and that other bacterial components are involved.

For ISR in radish triggered by WCS417r, the additional determinants were hypothesised to be iron regulated, since under conditions of low iron availability the LPS mutant did induce ISR (Leeman et al., 1996). For the ISR-inducing *P. putida* WCS358r, these iron-regulated bacterial determinants were hypothesised to be the pseudobactin-type siderophore of this strain. Application of purified pseudobactin or ferric–pseudobactin complex did induce systemic resistance against *Pst* DC3000. However, mutants defective in pseudobactin biosynthesis were as effective in inducing resistance as the wild-type strain. In addition, a double mutant of WCS358r that both lacked the O-antigenic side chain of the LPS, and was defective in pseudobactin biosynthesis, was as effective as the parental strain in inducing resistance. The involvement of the flagella was evaluated using both partly purified flagella and mutants of WCS358r that lack flagella. Again, a similar phenomenon was observed: flagella triggered ISR, but fla$^-$ mutants were equally effective (P.A.H.M. Bakker and I. Van der Sluis, unpublished results). This demonstrates that rhizobacteria-mediated ISR in Arabidopsis is a complex process that can involve several bacterial traits.

Signalling

A novel signalling pathway controlling induced systemic resistance in Arabidopsis

The signalling pathway controlling pathogen-induced SAR has been well studied in Arabidopsis. As in many other species, pathogen-induced SAR in Arabidopsis is effective against a broad-spectrum of pathogens and is tightly correlated with the activation of *PR* genes (Uknes et al., 1993; Cameron et al., 1994; Mauch-Mani and Slusarenko, 1994). Transgenic SA-nonaccumulating NahG plants fail to express SAR and *PR* genes, indicating that SA is required for the SAR signalling pathway (Lawton et al., 1995). Analysis of Arabidopsis mutants that are affected in their response to either jasmonic acid (JA) or ethylene develop normal levels of pathogen-induced SAR (Lawton et al., 1994; 1995; 1996), indicating that the SAR pathway functions independently of JA- and ethylene-dependent defence responses.

In contrast to pathogen-induced SAR, WCS417r-mediated ISR in Arabidopsis is independent of SA accumulation and *PR* gene activation, as SA-nonaccumulating NahG plants develop normal levels of ISR against *Pst* DC3000 after colonisation of the roots by WCS417r (Pieterse et al., 1996; Van Wees et al., 1997; Table 1). Similarly, the SA induction-deficient mutants *sid1-1* and *sid2-1* (Nawrath and Métraux, 1999) express WCS417r-mediated ISR (C.M.J. Pieterse, unpublished results; Table 1), again demonstrating that WCS417r-mediated ISR is SA-independent. Using the JA response mutant *jar1-1*, the ethylene response mutant *etr1-1* and the SAR regulatory mutant *npr1-1*, it was demonstrated that signal transduction leading to WCS417r-mediated ISR requires responsiveness to both JA and ethylene and, similar to pathogen-induced SAR, is dependent on NPR1 (Pieterse et al., 1998; Table 1). Like WCS417r, methyl jasmonate (MeJA) and the ethylene precursor 1-aminocyclopropane-1-carboxylate (ACC) were effective in inducing resistance against *Pst* DC3000 in SA-nonaccumulating NahG plants. Moreover, MeJA-induced protection was blocked in *jar1-1*, *etr1-1* and *npr1-1* plants, whereas ACC-induced protection was affected in *etr1-1* and *npr1-1* plants, but not in *jar1-1* plants. Hence, it was postulated that WCS417r-mediated ISR follows a novel signalling pathway in which components from the JA and ethylene response are successively engaged to trigger a defense reaction that, like SAR, is regulated by NPR1 (Pieterse et al., 1998). Downstream of NPR1, *PR* genes are activated in the SAR pathway but not in the ISR pathway (Cao et al., 1994; Pieterse et al., 1998). Evidently, NPR1 differentially regulates ISR- and SAR-related gene expression, depending on the pathway that is activated upstream of it.

Besides WCS417r, strain WCS358r has also been demonstrated to induce the SA-independent ISR pathway in Arabidopsis (Van Wees et al., 1997). Moreover, Press et al. (1997) found that the biological control strain *Serratia marcescens* 90-166 was able

to induce protection in both wild-type and transgenic NahG tobacco plants against *P. syringae* pv. *tabaci*, indicating that the ability to trigger an SA-independent pathway controlling systemic resistance is not uncommon among ISR-inducing rhizobacteria. But not all resistance-inducing rhizobacteria trigger a SA-independent resistance. For instance, *P. 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 et al., 1997; 1999a,b; Maurhofer et al., 1998).

Infiltration of leaves with ISR-inducing rhizobacteria triggers the ISR pathway

Infiltration of leaves with ISR-inducing *P. fluorescens* WCS417r bacteria induced protection against *Pst* DC3000 in non-infiltrated leaves (Pieterse et al., 1996). To test whether infiltration of leaves with ISR-inducing rhizobacteria triggers the same signalling pathway as root application, Arabidopsis genotypes Columbia, NahG, *jar1-1*, *etr1-1* and *npr1-1* were tested for their ability to express ISR against *Pst* DC3000 after pressure infiltrating 3 lower leaves with ISR-inducing WCS417r bacteria. Leaf infiltration and root application of WCS417r were similarly effective in eliciting ISR in wild-type Columbia plants. SA-nonaccumulating NahG plants also developed a significant level of ISR after leaf induction. In contrast, mutants *jar1-1*, *etr1-1* and *npr1-1* did not express ISR after infiltration of the leaves with ISR-inducing WCS417r bacteria (Pieterse et al., 2000a). Moreover, infiltration of 3 lower leaves per plant with WCS417r or WCS358r resulted in a significant level of protection against *Pst* DC3000 in the non-treated leaves, whereas WCS374r did not induce resistance. These results are in full agreement with those obtained after application of WCS417r, WCS358r or WCS374r bacteria to the roots (Pieterse et al., 1998; Van Wees et al., 1997), and demonstrate that ISR-inducing rhizobacteria trigger the same systemic signalling pathway when applied to either roots or leaves.

Rhizobacteria-mediated ISR requires ethylene-dependent signalling at the site of induction

Previously, Knoester et al. (1999) tested a set of well-characterised Arabidopsis mutants, that are affected at different steps in the ethylene-signalling pathway, for their ability to express ISR. None of the mutants developed ISR against *Pst* DC3000 after treatment of the roots with WCS417r (Table 1). Evidently, an intact ethylene-signalling pathway is required for the expression of ISR. Interestingly, mutant *eir1-1*, which is insensitive to ethylene in the roots only, was able to mount ISR when WCS417r was infiltrated in the leaves, but not when the bacteria were applied to the roots. If ethylene signalling was required only for systemic expression of ISR at the site of challenge inoculation, then *eir1-1* plants would be expected to develop normal levels of ISR in the leaves after application of WCS417r to the roots. However, this was not the case. It is therefore postulated that ethylene signalling is required at the site of application of the inducer, suggesting that ethylene is involved in the generation or translocation of the systemically transported ISR signal. This does not rule out the possibility that components of the ethylene response may also be required for the expression of ISR in tissues distant from the site of induction.

Rhizobacteria-mediated ISR and pathogen-induced SAR act additively

The plant signalling molecules SA and JA play an important role in induced disease resistance pathways. Cross-talk between defence signalling pathways has been demonstrated: JA and ethylene can act in concert in activating defence responses, whereas SA can suppress JA-dependent responses (Reymond and Farmer, 1998; Pieterse and Van Loon, 1999). Together with the fact that ISR and SAR share the regulatory factor NPR1, the question was raised as to what extent the JA-dependent ISR pathway and the SA-dependent SAR pathway interact. Recently, Van Wees et al. (2000) investigated possible interactions between both pathways. Interestingly, simultaneous activation of both pathways resulted in an additive effect on the level of induced protection against *Pst* DC3000. In Arabidopsis genotypes that are blocked in either SAR or ISR, this additive effect was not evident. Moreover, expression of the SAR marker gene *PR-1* was not altered in plants expressing both ISR and SAR compared to plants expressing SAR alone, indicating that the SAR and the ISR pathway are compatible and that there is no significant cross-talk between these signalling pathways. Furthermore, plants expressing both types of induced resistance did not show elevated levels of

Npr1 transcripts. Apparently, the constitutive level of NPR1 is sufficient to facilitate simultaneous expression of both SAR and ISR. This suggests that enhanced levels of protection were established through parallel activation of complementary, NPR1-dependent defense responses.

Expression

Search for rhizobacteria-mediated ISR-related genes

The state of pathogen-induced SAR is characterised 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). However, although some PRs possess anti-microbial activity, a causal relationship between accumulation of PRs and the broad-spectrum resistance characteristic of SAR has never been convincingly demonstrated (Van Loon, 1997). Of many defence-related genes tested in *Arabidopsis* (e.g. the SA-inducible genes *PR-1*, *PR-2* and *PR-5* and the ethylene- and/or JA-inducible genes *Hel*, *ChiB*, *Pdf1.2*, *Atvsp*, *Lox1*, *Lox2* and *Pal1*), none were found to be up-regulated in plants expressing ISR (Van Wees et al., 1999a). Moreover, neither standard differential screening of a cDNA library of WCS417r-induced plants, nor 2D-gel analysis of proteins from induced and non-induced plants yielded significant differences (Van Wees et al., 1999b). Thus, in contrast to SAR, the onset of ISR is not associated with major changes in gene expression. 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.

In another approach to search for ISR-related genes, a large collection of Arabidopsis lines containing enhancer-trap *Ds* transposons and the β-glucuronidase (GUS) reporter gene (Vroemen et al., 1998) were screened. One enhancer-trap line showed local GUS activity in the roots upon colonisation by WCS417r (K.M. Lèon-Kloosterziel, unpublished results). Interestingly, the roots of this line showed a similar expresssion pattern after treatment of the roots with the ethylene precursor ACC, indicating that this line contains a transposon insertion in the vicinity of an ethylene-inducible gene that is up-regulated in the roots upon colonisation by WCS417r. There are several candidate genes in the vicinity of the enhancer-trap *Ds* transposon. The identification of the putative ISR-related gene that is responsible for GUS expression is in progress and could provide more insight into the molecular mechanisms involved in rhizobacteria-mediated ISR.

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

In a genetic approach to identify ISR-related genes, 11 ecotypes of Arabidopsis were screened for their potential to express ISR and SAR against *Pst* DC3000 (Ton et al., 1999; Table 1). All ecotypes tested developed SAR. However, of the 11 ecotypes tested, RLD and Wassilewskija did not develop ISR after treatment of the roots with WCS417r. The WCS417r-nonresponsive phenotype was associated with a relatively high susceptibility to *Pst* DC3000, which was apparent as both a greater proliferation of the pathogen in the leaves and more severe disease symptoms. Genetic analysis of the F1, F2 and F3 progeny of a cross between the WCS417r-responsive ecotype Columbia and the WCS417r- nonresponsive ecotype RLD, revealed that both the potential to express ISR and the relatively high level of basal resistance against *Pst* DC3000 are monogenic, dominant traits that are genetically linked. The corresponding locus, designated *ISR1*, was mapped between cleared amplified polymorphic sequence (CAPS) markers *B4* and *GL1* on chromosome III. Neither responsiveness to WCS417r, nor the relatively high level of basal resistance against *Pst* DC3000 was complemented in the F1 progeny of crosses between RLD and Wassilewskija, indicating that both ecotypes are affected in the same locus.

Interestingly, mutants *jar1-1* and *etr1-1*, that are affected in their response to JA and ethylene, respectively, showed the same phenotype as ecotypes RLD and Wassilewskija in that they were both unable to express WCS417r-mediated ISR and showed enhanced susceptibility to *Pst* DC3000 infection (Pieterse et al., 1998). Analysis of ethylene-responsiveness of RLD and Wassilewskija revealed that both ecotypes showed a reduced sensitivity to ethylene, that co-segregated with the recessive alleles of the *ISR1* locus (Ton et al., 2000). Therefore, it is proposed that the Arabidopsis *ISR1* locus encodes a novel component of the ethylene-response pathway that plays an important role in disease-resistance signalling.

Production of JA and ethylene during rhizobacteria-mediated ISR

Increased production of JA and ethylene is an early symptom of active defence in infected plants (De Laat and Van Loon, 1982; Gundlach et al., 1992; Mauch et al., 1994). Both signalling molecules co-ordinate the activation of a large set of defence responses, and when applied exogenously, can induce resistance themselves (Boller, 1991; Cohen et al., 1993; Pieterse et al., 1998). In Arabidopsis, both JA and ethylene activate specific sets of defence-related genes and resistance against *Pst* DC3000 (Pieterse et al., 1998; Van Wees et al., 1999). Recently, Van Wees et al. (1999a) monitored the expression of a set of well-characterised JA- and/or ethylene-responsive genes (i.e. *Lox1*, *Lox2*, *Atvsp*, *Pdf1.2*, *Hel*, *ChiB* and *Pal1*) in Arabidopsis plants expressing 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 WCS417r-mediated ISR in Arabidopsis was not associated with major changes in the levels of either JA or ethylene. Indeed, analysis of local and systemic levels of JA and ethylene revealed that WCS417r-mediated ISR is not associated with changes in the production of these signal molecules (Pieterse et al., 2000a). By using the *Lox2* co-suppressed transgenic line S-12, we 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), expressed normal levels of ISR, irrespective of whether ISR was induced via roots or leaves (Pieterse et al., 2000a; Table 1). Together, these results suggest that the JA and ethylene dependency of ISR is based on enhanced sensitivity to these hormones, rather than on an increase in their production.

Potentiation of JA-responsive genes in plants expressing rhizobacteria-mediated ISR

If the JA and ethylene 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 pathogen-induced JA or ethylene production. Therefore, the expression of the JA-responsive genes *Atvsp*, *Pdf1.2*, *Lox2* and *Pal1*, the ethylene-responsive genes *Hel* and *ChiB* and the SA-inducible genes *PR-1*, *PR-2* and *PR-5* was analysed after challenge of control, SAR- and ISR-expressing plants (Van Wees et al., 1999a). Infection with *Pst* DC3000 induced the expression of all genes tested. In challenged, SAR-expressing plants the SA-inducible genes *PR-1*, *PR-2* and *PR-5* showed a potentiated expression compared to challenged control plants. In challenged, ISR-expressing plants, only *Atvsp* displayed an enhanced level of expression in comparison to challenged control plants. The expression of the other JA-responsive genes was not potentiated, suggesting that ISR is associated with the potentiation of a specific set of JA-responsive genes.

Rhizobacteria-mediated ISR is associated with enhanced capacity for conversion of ACC to ethylene

In higher plants, ethylene is produced from methionine (Met) via S-adenosyl-L-methionine (SAM) and ACC (Met → SAM → ACC → ethylene; Kende, 1993; Kende and Zeevaart, 1997; Theologis, 1992). The last two steps of this biosynthetic pathway are catalysed by ACC synthase and ACC oxydase, respectively. Pathogen infections leading to chlorotic or necrotic symptoms caused an increase in ethylene production with ACC synthase and ACC oxidase activity being increased sequentially (De Laat and Van Loon, 1982). Under normal conditions the conversion of SAM to ACC is the rate-limiting step, however, during infections, ACC accumulates transiently, indicating that ACC oxidase activity restricts ethylene production. Previously, it was demonstrated that the capacity for converting ACC to ethylene was increased systemically in SAR-expressing tobacco plants (De Laat and Van Loon, 1983), providing a greater capacity for producing ethylene after challenge inoculation.

In Arabidopsis, ethylene production in systemic tissues expressing either ISR or SAR is not increased compared to non-induced plants. However, after treatment with a saturating dose of 1 mM ACC, ISR-expressing plants showed a statistically significant higher level of ethylene production than ACC-treated control plants, irrespective of whether ISR was induced by WCS417r treatment of the roots or by leaf infiltration (Pieterse et al., 2000a). The magnitude of the increase in ACC-converting capacity varied from 20% to 50% between experiments and was similar to that observed in SAR-expressing plants. Evidently, the capacity to convert ACC to ethylene is increased in Arabidopsis plants expressing either ISR or SAR, providing a greater potential for producing ethylene

upon pathogen attack. As application of ACC has been shown to induce resistance against *Pst* DC3000 in Arabidopsis (Pieterse et al., 1998), a faster or greater production of ethylene in the initial phase of infection may contribute to enhanced resistance against this pathogen. The significance of this phenomenon needs further investigation.

Conclusions and future prospects

Elucidation of plant signalling pathways controlling expression of disease resistance is a major objective in investigations of plant–pathogen interactions. Recent advances in research on plant defence signalling pathways have shown that plants are capable of

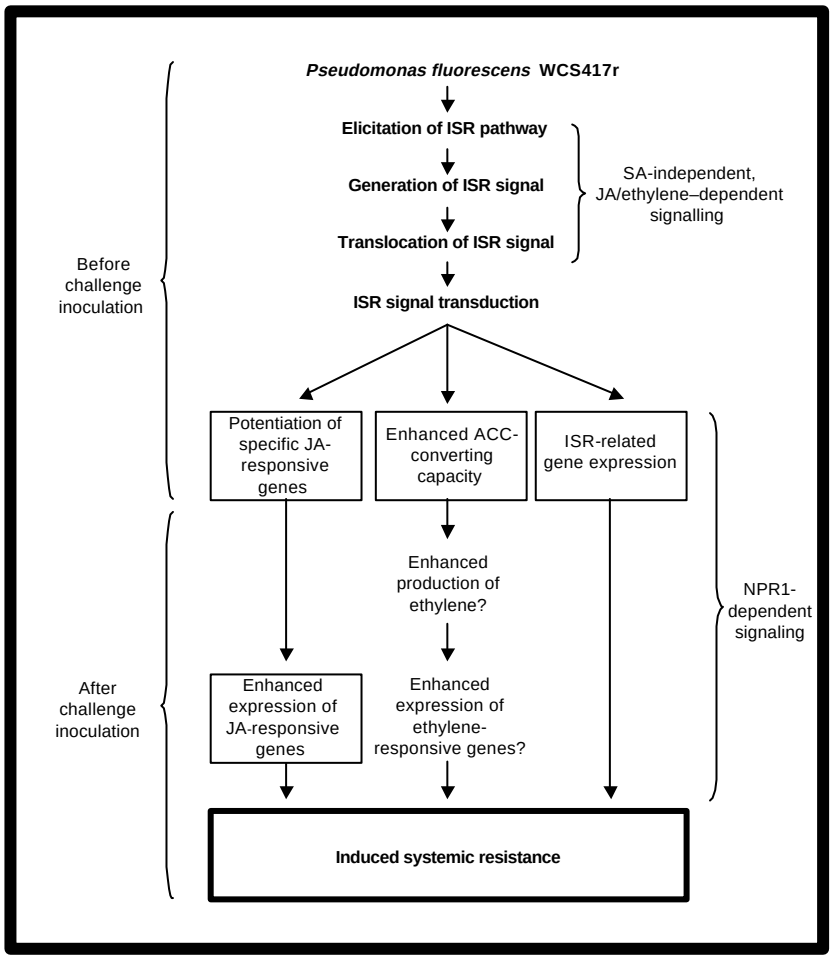

Figure 1. Working model explaining the possible involvement of JA and ethylene in *P. fluorescens* WCS417r-mediated ISR in Arabidopsis. Colonisation of Arabidopsis roots by WCS417r triggers a signalling pathway that, unlike the pathogen-induced SAR pathway, is independent of SA and is not associated with the activation of *PR* genes, but instead requires responsiveness to both JA and ethylene. In the ISR pathway, components from the JA and the ethylene response are successively engaged, leading to a systemic resistance that, like SAR, is regulated by NPR1. Ethylene responsiveness is required at the site of ISR induction, suggesting that this signal molecule is involved in the generation or translocation of the systemically transported signal. However, this does not rule out the possibility that JA and/or ethylene are also involved in later stages of the ISR pathway. ISR is neither locally, nor systemically accompanied by an increase in JA or ethylene biosynthesis. Accordingly, Arabidopsis plants show no increase in JA- or ethylene-responsive gene expression in response to induction of ISR. However, a specific set of JA-responsive genes is potentiated in ISR-expressing plants, leading to a higher level of expression after challenge inoculation. Moreover, ISR-expressing plants have a higher capacity to convert ACC to ethylene, providing a greater potential to produce ethylene upon challenge. Both phenomena suggest that ISR-expressing plants are primed to express JA- and/or ethylene-dependent defence reactions faster or to a higher level after attack by a challenging pathogen.

differentially activating distinct defence pathways, depending on the type of invader encountered (reviewed by Bostock, 1999; Dong, 1998; Glazebrook, 1999; Maleck and Dietrich, 1999; Mauch-Mani and Métraux, 1998; Pieterse and Van Loon, 1999; Reymond and Farmer, 1998). SA is an important signalling molecule in both locally and systemically induced resistance responses. However, research on rhizobacteria-mediated ISR signalling in Arabidopsis demonstrated that JA and ethylene play key the roles (summarised in Figure 1). During the past 5 years, research on rhizobacteria-mediated ISR has increased our knowledge of the molecular mechanisms involved in this form of induced disease resistance. An important conclusion is that different rhizobacteria utilise different mechanisms for triggering systemic resistance: some rhizobacteria trigger a SA-dependent pathway, others a JA/ethylene-dependent pathway, and additional pathways are likely to be discovered in the near future. In this respect, it is interesting to note that simultaneous activation of the SA-dependent SAR pathway and the JA/ethylene- dependent ISR pathway resulted in an additive effect on the level of induced resistance attained (Van Wees et al., 2000). Therefore, combining rhizobacterial strains that trigger different signalling pathways in the plant provides an attractive possibility for the improvement of disease control.

In contrast to SAR, rhizobacteria-mediated ISR in Arabidopsis is not associated with major changes in gene expression. Currently, research on the molecular mechanisms underlying ISR is hampered by the lack of reliable molecular markers. Therefore, future research will be focussed on identifying such marker genes using techniques such as screening of DNA microarrays, screening of enhancer/gene-trap lines, and map-based cloning approaches. Furthermore, the mechanisms involved in potentiation of JA-responsive gene expression and the increased ACC-converting capacity in ISR-expressing plants need to be investigated. Both latter findings are examples of priming that may lead to a faster and/or enhanced activation of JA- and ethylene-dependent defence reactions upon attack by a challenging pathogen. If priming of defence responses plays an important role in ISR, then this could explain the absence of major changes in defence-related gene expressing prior to challenge. Investigations of these phenomena will be most challenging and will certainly provide more insight in the molecular mechanisms of induced disease resistance.

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