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Review article

Induced Systemic Resistance by Plant Growth-Promoting Rhizobacteria

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Abstract

Rhizobacteria are present in large numbers on the root surface, where plant exudates and lysates provide nutrients. Selected strains of beneficial, plant growth-promoting rhizobacteria (PGPR) trigger a plant-mediated induced systemic resistance (ISR) response that is effective against a broad spectrum of plant pathogens. To study the molecular basis of ISR, an *Arabidopsis thaliana*-based model was developed, using PGPR strain *Pseudomonas fluorescens* WCS417r as the inducing agent. Genetic dissection of the ISR signalling pathway revealed that ISR is regulated by a defence pathway in which the phytohormones jasmonic acid and ethylene play key roles.

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Interestingly, the state of ISR is not associated with major changes in gene expression. Instead, ISR-expressing plants are primed to activate specific sets of defense-related genes faster or to a higher level upon pathogen attack. Here we review the current state of knowledge of the signal transduction steps involved in the ISR pathway in *Arabidopsis* that leads from recognition of the rhizobacteria by the roots to systemic expression of broad-spectrum disease resistance in above-ground foliar tissues.

Keywords: *Arabidopsis*, ethylene, jasmonic acid, induced plant defence, *Pseudomonas fluorescens*

1. Introduction

Selected strains of non-pathogenic, rhizosphere-colonising bacteria are referred to as plant growth-promoting rhizobacteria (PGPR), because they can stimulate growth of the plant (Kloepper et al., 1980). Growth promotion results mainly from suppressing soil-borne pathogens and other deleterious micro-organisms (Schippers et al., 1987), but also direct effects on plant growth have been reported (Lynch, 1976; Van Peer and Schippers, 1989). 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). The biological control activity of selected *Pseudomonas* spp. strains are effective under field conditions (Tuzun and Kloepper, 1995; Wei et al., 1996) and in commercial greenhouses (Leeman et al., 1995b), and can be the result of competition for nutrients, siderophore-mediated competition for iron, or antibiosis (Bakker et al., 1991).

Apart from a direct antagonistic effect on soil-borne pathogens, some PGPR strains are also able to reduce disease in above-ground plant parts through a plant-mediated mechanism called induced systemic resistance (ISR) (Van Loon et al., 1998). PGPR-mediated ISR has been demonstrated in many plant species, e.g. bean, carnation, cucumber, radish, tobacco, tomato and the model plant *Arabidopsis thaliana*, and is effective against a broad spectrum of plant pathogens, including fungi, bacteria and viruses (Van Loon et al., 1998). Phenotypically, PGPR-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).

2. Rhizobacteria-Mediated ISR in *Arabidopsis*

To study rhizobacteria-mediated ISR, an *Arabidopsis*-based model system was developed. In this model system, the non-pathogenic rhizobacterial strain *Pseudomonas fluorescens* WCS417r is used as the inducing agent (Pieterse et al., 1996). WCS417r has been shown to trigger ISR in several plant species, e.g. carnation, radish, tomato, and bean (Pieterse et al., 2001b), and promotes plant growth in *Arabidopsis* in the absence of a pathogen (Pieterse and Van Loon, 1999). Colonisation of Arabidopsis roots by ISR-inducing WCS417r bacteria protects the plants against different types of pathogens, including the bacterial leaf pathogens *Pseudomonas syringae* pv. *tomato* DC3000 (*Pst* DC3000) 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; Ton et al., 2002b; Van Wees et al., 1997). 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.

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) 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 WCS358r, whereas radish and carnation are not (Leeman et al., 1995a; Van Peer et al., 1991; Van Peer and Schippers, 1992; 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 (Ton et al., 1999; Van Wees et al., 1997). 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.

3. 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; Van Loon et al., 1998). To

compare the spectrum of effectiveness of ISR and SAR, a range of viral, bacterial, fungal and oomycete pathogens of *Arabidopsis* were tested. Both WCS417r-mediated ISR and SAR induced by an avirulent strain of the pathogen *Pst* DC3000 appeared to be effective against bacterial speck and black rot disease caused by the bacterial pathogens *Pst* DC3000 and *X. campestris* pv. *armoraciae*, respectively (Pieterse et al., 1996; Ton et al., 2002b). 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; Van Wees et al., 1997). Moreover, disease caused by the downey mildew pathogen *P. parasitica* was inhibited in both cases, although SAR was significantly more effective than ISR (Ton et al., 2002b). 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., 2002b). 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.

4. ISR and SAR are Regulated by Distinct Signalling Pathways

Early research on molecular mechanisms involved in pathogen-induced SAR showed that the onset of SAR is accompanied by a local and systemic increase in the endogenous levels of SA (Malamy et al., 1990; Métraux et al., 1990) and the concomitant up-regulation of a large set of genes (Ward et al., 1991), including ones encoding pathogenesis-related (PR) proteins (Van Loon and Van Strien, 1999). Several PR proteins possess antimicrobial activity and are thought to contribute to the state of resistance attained. 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), demonstrating that SA is both necessary and sufficient for induction of SAR (Ryals et al., 1996). 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; Delaney et al., 1995). This gene was designated *npr1* (for non-expresser of *PR* genes), or *nim1* (for no 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 a SAR response, indicating that NPR1 functions downstream of SA in the SAR pathway. The *NPR1* gene encodes a protein with ankyrin-like repeats, which are known to mediate protein-

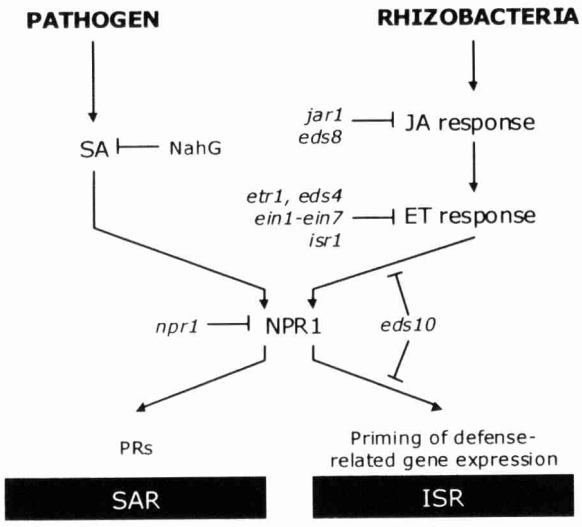

Figure 1. Schematic model describing the pathogen-induced SAR and the rhizobacteria-mediated ISR signal transduction pathways in *Arabidopsis* (see text for details).

protein interactions and are present in proteins with diverse functions (Cao et al., 1997; Ryals et al., 1997). Recently, evidence was provided demonstrating that, upon induction of SAR, NPR1 is translocated to the nucleus, 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 (Kinkema et al., 2000; Subramaniam et al., 2001; Zhang et al., 1999). In Fig. 1 the main characteristics of the SAR signalling pathway are depicted.

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 WCS417r did not accumulate PR proteins, although these plants clearly showed enhanced resistance against fusarium wilt disease (Hoffland et al., 1995). Similarly, *Arabidopsis* plants expressing WCS417r-mediated ISR showed enhanced resistance against *F. oxysporum* f.sp. *raphani* and *Pst* DC3000, but this did not coincide with the activation of the SAR marker genes *PR-1*, *PR-2*, and *PR-5* (Pieterse et al., 1996; Van Wees et al., 1997). Determination of SA levels in ISR-expressing *Arabidopsis* plants revealed that ISR is not associated with increased accumulation of SA (Pieterse et al., 2000). Moreover, WCS417r-mediated ISR was normally expressed in SA-

nonaccumulating *Arabidopsis* NahG plants (Pieterse et al., 1996; Van Wees et al., 1997). This led to the conclusion that WCS417r-mediated ISR is a SA-independent resistance response, and that rhizobacteria-mediated ISR and pathogen-induced SAR are regulated by distinct signalling pathways.

Apart from WCS417r, strain WCS358r has also been demonstrated to induce the SA-independent ISR pathway in *Arabidopsis* (Van Wees et al., 1997). 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). In wild-type and NahG tomato plants, a similar SA-independent resistance was observed against *Phytophthora infestans* after treatment of the roots with the PGPR strains *Bacillus pumilus* SE34 and *Pseudomonas fluorescens* 89B61 (Yan et al., 2002). All together, this indicates 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 a 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; Maurhofer et al., 1998).

5. Genetic Dissection of the ISR Signalling Pathway

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; Pieterse et al., 2001a). 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 on their ability to express ISR. Both mutants were unable to mount resistance against *Pst* DC3000 after colonisation of the roots by *P. fluorescens* WCS417r (Pieterse et al., 1998), indicating that ISR requires responsiveness to both JA and ET. In addition to *etr1-1*, a set of other well-characterised *Arabidopsis* mutants that are affected at different steps in the ET-signalling pathway were tested for their ability to express ISR. None of the mutants developed ISR against *Pst* DC3000 (Knoester et al., 1999), 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 WCS417r, MeJA and ACC were effective in inducing resistance against *Pst* DC3000 in SA-nonaccumulating 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 WCS417r-mediated ISR follows a signalling pathway in which components from the JA and ET response are successively engaged (Pieterse et al., 1998).

NPR1 has been shown to be an important regulatory factor in the SA-dependent SAR response (Cao et al., 1994). To investigate whether NPR1 is involved in the SA-independent ISR response as well, *Arabidopsis* mutant *npr1* was tested. Surprisingly, mutant *npr1* plants were blocked in their ability to express WCS417r-mediated ISR, indicating that, like pathogen-induced SAR, rhizobacteria-mediated ISR is an NPR1-dependent defence response (Pieterse et al., 1998). 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 suggests that NPR1 is able to differentially regulate defence gene expression, depending on the signalling pathway that is activated upstream of it. In Fig. 1 the main characteristics of the ISR signalling pathway are depicted.

6. Identification of the *Arabidopsis ISR1* Locus

In a genetic approach to identify novel components from the ISR signalling pathway, 10 *Arabidopsis* ecotypes were screened for their potential to express ISR against *Pst* DC3000 (Ton et al., 1999). Of the 10 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 the development of more severe disease symptoms. Genetic analysis of the 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 on chromosome III (Ton et al., 1999) and was

shown to be required for ISR against different pathogens (Ton et al., 2002c).

Interestingly, mutants *jar1-1* and *etr1-1*, that are affected in their response to JA and ET, 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 infection by *Pst* DC3000 (Pieterse et al., 1998). Analysis of the ET-responsiveness of RLD and Wassilewskija revealed that both ecotypes have a reduced sensitivity to ET, that co-segregates with the recessive alleles of the *ISR1* locus (Ton et al., 2001). 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.

7. The Role of Jasmonic Acid and Ethylene in ISR

In *Arabidopsis*, both JA and ET activate specific sets of defence-related genes and when applied exogenously they confer resistance against *Pst* DC3000 (Pieterse et al., 1998; Van Wees et al., 1999). To investigate whether ISR is associated with changes in JA/ET-responsive gene expression, Van Wees et al. (1999) monitored the expression of a set of well-characterised JA- and/or ET-responsive genes (i.e. *LOX1*, *LOX2*, *VSP*, *PDF1.2*, *HEL*, *CHI-B*, 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 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 WCS417r-mediated ISR is not associated with changes in the production of these signal molecules (Pieterse et al., 2000). This suggests that the JA and ET dependency of ISR is based on enhanced sensitivity to these hormones, rather than on an increase in their production.

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 *Pst* DC3000 compared to inoculated control plants (Van Wees et al., 1999). In the same study, several other JA-responsive genes were tested as well, 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 of a specific set of JA-responsive genes. Priming of defence-related genes, leading to a faster and/or higher level of expression

after challenge inoculation, emerged as a common feature of different types of induced resistance (Conrath et al., 2002). It 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 unknown but is one of the challenges for future research.

8. 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 came 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; Glazebrook, 2001). 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 turnip crinkle virus seems to be controlled predominantly by a SA-dependent pathway. Only SA-nonaccumulating NahG plants exhibit enhanced disease susceptibility to these pathogens (Delaney et al., 1994; Kachroo et al., 2000), whereas mutants affected in JA or ET signalling do not (Kachroo et al., 2000; Thomma et al., 1998). In contrast, basal resistance against the fungal pathogens *A. brassicicola* and *B. cinerea* is reduced only in JA- and ET-insensitive mutants, and not in NahG plants (Thomma et al., 1998; Thomma et al., 1999). Interestingly, basal resistance against the bacterial pathogens *Pst* DC3000 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; Ton et al., 2002b), suggesting that basal resistance against these pathogens is controlled by a combined action of SA, JA and ET. Comparison of the effectiveness of SA-dependent SAR and JA/ET-dependent ISR against these different *Arabidopsis* pathogens, revealed that SAR is predominantly effective against pathogens that in non-induced plants are resistant through SA-dependent basal resistance mechanisms, whereas ISR is predominantly effective against pathogens that in non-induced plants are resistant through JA/ET-dependent basal resistance responses (Ton et al., 2002b). 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.

9. Analysis of Enhanced Disease Susceptibility Mutants

Because of the association between induced resistance and basal resistance, we made use of a collection of *Arabidopsis eds* mutants with 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 *Pst* DC3000. Out of 11 *eds* mutants tested, *eds4-1*, *eds8-1*, and *eds10-1* were non-responsive to induction of ISR by WCS417r (Ton et al., 2002a). 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*). Moreover, *eds4-1* and *eds8-1* showed 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 (Ton et al., 2002a). Future research should reveal the exact role of these signalling components in the expression of ISR.

10. The Hunt for ISR-Related Genes

Over the past years, several approaches have been initiated to identify ISR-related gene expression. In one of the approaches, we screened a large collection of *Arabidopsis* lines containing enhancer-trap *Ds* transposons and the β-glucuronidase (*GUS*) reporter gene with minimal promoter (Vroemen et al., 1998). One enhancer-trap line showed local GUS activity in the roots upon colonization with WCS417r. This local GUS expression was not observed after treatment of the roots with *Escherichia coli*, indicating that the induction was *Pseudomonas* specific (Léon-Kloosterziel et al., 2002). Interestingly, a similar expression pattern was observed after treatment of the roots with the ET precursor ACC, indicating that this line contains a transposon insertion in the vicinity of an ET-inducible gene that is up-regulated upon colonization with WCS417r. There are several candidate genes in the vicinity of the enhancer-trap *Ds* transposon, one of which encodes a thaumatin-like protein. Gene expression analyses confirmed that this thaumatin-like gene is up-regulated in response to treatment of the roots with WCS417r or ACC. Analysis of the role of the thaumatin-like gene in ISR might provide more insight into the molecular mechanisms involved in rhizobacteria-mediated ISR.

In another approach, the expression pattern of a large set of known, well-

characterised defence-related genes of *Arabidopsis* was analysed upon induction of ISR by WCS417r. This set of genes consisted of 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*. However, none of the genes tested were found to be up-regulated in plants expressing ISR, neither locally in the roots, nor systemically in the leaves (Van Wees et al., 1999). Currently, we are analysing transcript profiles of over 8000 Arabidopsis genes using Affymetrix GeneChip Arabidopsis Genome Arrays. Preliminary data confirm that the onset of ISR is not associated with major changes in gene expression (Verhagen et al., 2001). This is clearly in contrast to the onset of pathogen-induced SAR, in which *PR*-gene products accumulated systemically to levels from 0.3 to 1% of the total mRNA and protein content (Lawton et al., 1995). Nevertheless, ISR-expressing plants are clearly more resistant to different types of pathogens. Therefore, plants must possess as yet undiscovered defence mechanisms that contribute to broad-spectrum disease resistance. As mentioned above, priming of defence-related gene expression might 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 role of priming in the expression of rhizobacteria-mediated ISR is currently under investigation.

11. Combining ISR and SAR to Improve Biocontrol of Plant Diseases

Induced disease resistance is an attractive 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 (Van Loon et al., 1998). Therefore, detailed knowledge on 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 the ISR and the SAR pathway results in an enhanced level of induced protection against *Pst* DC3000 (Van Wees et al., 2000). This indicates that the JA/ET-dependent ISR pathway and the SA-dependent SAR pathway act independently and additively on the level of protection against this pathogen. Moreover, we provided evidence that ISR and SAR confer differential protection against different types of pathogens (Ton et al., 2002b). 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. Therefore, integrating both forms of induced resistance has great potential for future agricultural practices.

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