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Rhizobacteria-Mediated Induced Systemic Resistance (ISR) in *Arabidopsis*: Involvement of Jasmonate and Ethylene

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The capacity of a plant to express a broad-spectrum systemic acquired resistance (SAR) after primary infection is well known and extensively studied (Ryals *et al.*, 1996). A relatively unknown form of induced disease resistance is triggered by nonpathogenic, root-colonizing rhizobacteria and is commonly referred to as rhizobacteria-mediated induced systemic resistance (ISR; van Loon *et al.*, 1998). Rhizosphere bacteria are present in large numbers on the root. Certain strains stimulate plant growth (Fig. 1) and are therefore called plant growth-promoting rhizobacteria (PGPR). Selected strains with biological control activity, mainly fluorescent *Pseudomonas* spp., reduce plant diseases by suppressing soil-borne pathogens through competition for nutrients, siderophore-mediated competition for iron or antibiosis (Bakker *et al.*, 1991). Some of these strains reduce disease through a plant-mediated mechanism that is phenotypically similar to SAR, as the induced resistance extends to the above-ground plant parts and is effective against different types of pathogens (van Loon *et al.*, 1998). Some rhizobacteria trigger the salicylic acid (SA)-dependent SAR pathway by producing SA at the root surface (de Meyer *et al.*, 1997). In other cases, rhizobacteria trigger a different signaling pathway that does not require SA (Pieterse *et al.*, 1996; van Wees *et al.*, 1997; Press *et al.*, 1997).

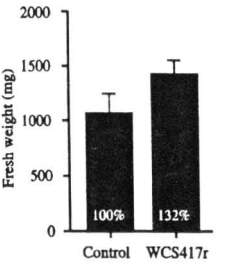

Fig. 1. Plant growth-promoting effect on *Arabidopsis* grown in soil containing *Pseudomonas fluorescens* WCS417r.

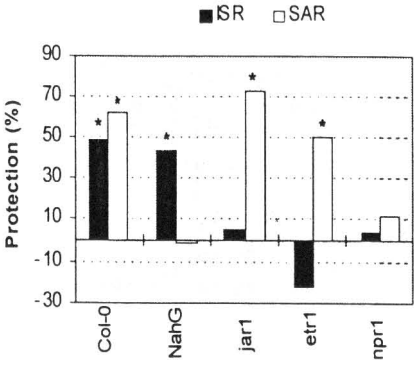

Fig. 2. Level of protection (= reduction in disease symptoms) against *Pst* in different *Arabidopsis* genotypes 4 days after challenge. ISR was induced by growing plants in soil containing WCS417r bacteria. SAR was induced by infiltrating 3 lower leaves per plant with avirulent *Pst*(*avrRpt2*) 3 days before challenge. Asterisks indicate statistically significant differences compared to control (set at 0 % protection) (Fisher's LSD test, α=0.05).

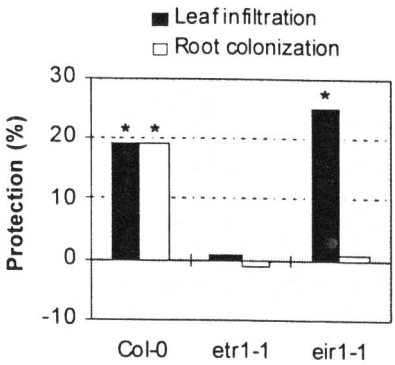

Fig. 3. Level of protection against *Pst* in *Arabidopsis* Col-0 plants and the ethylene response mutants *etr1-1* and *eir1-1*, 4 days after challenge. *eir1-1* displays ethylene insensitivity in the roots only. ISR was induced by growing plants in soil containing WCS417r or by infiltrating 3 lower leaves per plant with WCS417r 3 days before challenge. Asterisks indicate statistically significant differences compared to control (LSD test, α=0.05).

A Novel Signaling Pathway Controlling Induced Systemic Resistance

In *Arabidopsis, Pseudomonas fluorescens* WCS417r-mediated ISR has been demonstrated against *Pseudomonas syringae* pv. *tomato* DC3000 (*Pst*), *Fusarium oxysporum* and *Peronospora parasitica*. ISR is expressed as a reduction of symptoms and inhibition of pathogen growth. In contrast to SAR, this rhizobacteria-mediated ISR is independent of SA accumulation and pathogenesis-related (*PR*) gene activation (Pieterse *et al.*, 1996; van Wees *et al.*, 1997). Using the jasmonate (JA) response mutant *jar1*, the ethylene response mutant *etr1*, and the SAR regulatory mutant *npr1*, it was demonstrated that ISR signaling requires components of the JA and the ethylene response and, like SAR, depends on the regulatory protein NPR1 (Fig. 2). Downstream of NPR1, *PR* genes are activated in the SAR pathway (Cao *et al.*, 1994) but not in the ISR pathway (Pieterse *et al.*, 1998). Evidently, NPR1 differentially regulates ISR- and SAR-related gene expression depending on the pathway that is activated upstream.

ISR Requires Ethylene-Dependent Signaling at the Site of Induction

A set of *Arabidopsis* mutants that are affected at different steps in the ethylene signaling pathway were tested. None of the mutants developed ISR against *Pst* after treatment of the roots with WCS417r (Knoester *et al.*, 1999). Evidently, an intact ethylene signaling pathway is required for ISR. 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 (Fig. 3). If ethylene signaling were required only for systemic expression of ISR, then one would expect *eir1-1* plants to develop ISR in the leaves after application of WCS417r to the roots. However, this was not the case. We therefore postulate that ethylene signaling 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 ethylene responsiveness is also required for the expression of ISR in tissue distant from the site of induction.

ISR and SAR Are Additive

Cross-talk between defense signaling pathways has been demonstrated; JA and ethylene often act in concert in activating defense responses, whereas SA can suppress A-dependent responses (Raymond and Farmer, 1998; Pieterse and van Loon, 1999). Because ISR and SAR share the regulatory factor NPR1, the question was raised to what extent the ISR and SAR pathways interact. Simultaneous activation of both pathways resulted in an additive effect on the level of induced protection against *Pst* (Fig. 4). No enhanced level of protection was evident in *Arabidopsis* genotypes NahG, *jar1*, *etr1* and *npr1* that are blocked in either the ISR and/or the SAR pathway. Expression of the SAR marker gene *PR-1* was not altered in plants expressing both ISR and SAR compared to plants expressing SAR alone. Evidently, the additive effect on the level of protection is established through parallel activation of complementary defense responses that are both active against *Pst*. The fact that neither SAR nor ISR is inhibited when

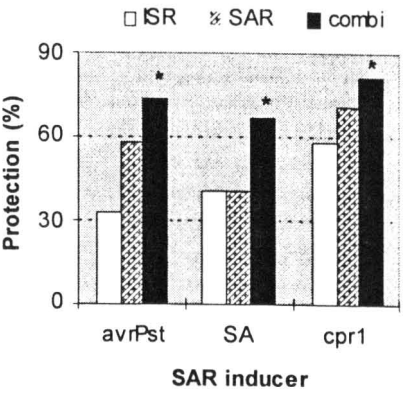

Fig. 4. Additive effects of ISR and SAR on the level of protection against *Pst*. ISR was induced by growing plants in soil containing WCS417r bacteria. SAR was either constitutively expressed as in mutant *cpr1*, or induced 3 days before challenge by infiltration of 3 lower leaves with *Pst*(*avrRpt2*) or spraying with 2.5 mM SA. Asterisks indicate statistically significant increases in the level of protection compared to either ISR or SAR alone (LSD test, α=0.05).

activated simultaneously, demonstrates that the ISR pathway and the SAR pathway are compatible.

JA, Ethylene and SA Production

To assess whether ISR is associated with increased production of either JA, ethylene or SA, we monitored the levels of these molecules in ISR-expressing plants. In contrast to pathogen-induced SAR, induction of ISR was not associated with an increased production of any of these molecules. Consistent with these findings, we did not observe induced expression of the JA-, ethylene- or SA-responsive genes *Atvsp, Pdf1.2, Lox2, Pal1, Hel, ChiB, PR-1, PR-2* and *PR-5* in ISR-expressing plants. After challenge with *Pst,* JA and ethylene production increased to the same extent in control and ISR-expressing plants. 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 Gene Expression

To investigate whether in ISR-expressing plants pathogen-induced JA or ethylene production leads to earlier or stronger defense responses, we analyzed 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* after challenge of control, SAR- and ISR-expressing plants. Pathogen infection induced the expression of all genes tested. In challenged, SAR-expressing plants the SA-inducible genes *PR-1, PR-2* and *PR-5* showed an enhanced expression compared to challenged control plants. In challenged, ISR-expressing plants, only the *Atvsp* gene was potentiated in comparison to challenged control plants. The expression of the other jasmonate-responsive genes was not enhanced, suggesting that ISR is associated with the potentiation of a specific set of jasmonate-responsive genes.

Identification of a Novel Locus (ISR1) Controlling ISR

Ten ecotypes of *Arabidopsis* were screened for their potential to express ISR and SAR against *Pst.* All ten ecotypes developed SAR after predisposal infection with avirulent *Pst(avrRpt2).* Eight of these developed ISR after treatment of the roots with WCS417r, but ecotypes RLD and Ws-0 did not (Fig. 5). This WCS417r-nonresponsive phenotype was associated with a relatively high susceptibility to *Pst,* which was apparent as both a higher proliferation of the pathogen in the leaves and more severe symptoms. Genetic analysis of the F_1, F_2 and F_3 progeny of a cross between the WCS417r-responsive ecotype Col-0 and the nonresponsive ecotype RLD, revealed that both the potential to express ISR and the relatively high

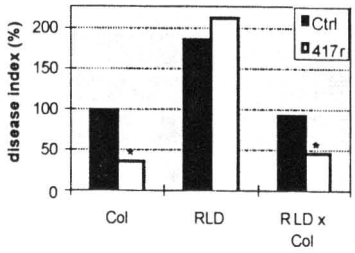

Fig. 5. Quantification of WCS417r-mediated ISR against *Pst* in ecotypes Col-0 and RLD and the F_1 progeny of a RLDxCol-0 cross. The disease index is the proportion of leaves with symptoms relative to control-treated Col-0 plants (which was set at 100%). Asterisks indicate statistically significant differences compared to the control treatment (LSD test, α=0.05).

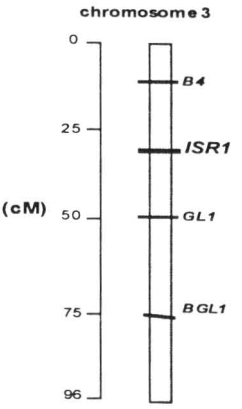

Fig. 6. Map position of the *ISR1* locus.

level of basal resistance against *Pst* are monogenic, dominant traits that are genetically linked. The corresponding locus, designated *ISR1*, was mapped between CAPS markers *B4* and *GL1* on chromosome 3 (Ton *et al.*, 1999; Fig. 6). Neither responsiveness to WCS417r, nor basal resistance against *Pst* were complemented in the F_1 progeny of crosses between RLD and Ws-0, indicating that both ecotypes are affected at the *ISR1* locus. We postulate that the *ISR1* locus controls a basal resistance response against *Pst* that is enhanced upon induction of ISR.

Search for ISR-Related Genes

SAR is associated with the activation of a large set of SAR genes (Ward *et al.*, 1991). Screens for gene expression and protein accumulation demonstrated that ISR is not associated with major changes in gene expression or protein abundance as observed during SAR. A screen of a collection of *Arabidopsis* lines containing enhancer trap *Ds* transposons and the GUS reporter gene yielded a line that shows GUS activity in the roots upon colonization by WCS417r. Interestingly, this line showed a similar expression pattern after treatment of the roots with the ethylene precursor ACC. Hence, this line contains a transposon insertion in the vicinity of an ethylene-inducible gene that is upregulated in the roots upon colonization by WCS417r. Cloning of this gene and of the *ISR1* gene mentioned above is in progress and could provide more insight into the molecular mechanisms involved in rhizobacteria-mediated ISR.

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