The role of ethylene in rhizobacteria-induced systemic resistance (ISR)

Pieterse, C.M.J.*, Van der Ent, S., Van Pelt, J.A. and Van Loon, L.C.

Section Phytopathology, Institute of Environmental Biology, Faculty of Science, Utrecht University, P.O. Box 800.84, 3508 TB Utrecht, The Netherlands. Phone +31 30 253 6887; Fax +31 30 251 8366. (*Corresponding author: C.M.J.Pieterse@bio.uu.nl)

1. Introduction

To protect themselves from disease, plants have evolved sophisticated defense mechanisms in which the signal molecules salicylic acid (SA), jasmonic acid (JA) and ethylene (ET) often play crucial role (Pieterse and Van Loon, 1999). Elucidation of signaling 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 (Durrant and Dong, 2004). Plants of which the roots have been colonized by specific strains of nonpathogenic fluorescent *Pseudomonas* spp. develop a phenotypically similar form of protection that is called rhizobacteria-mediated induced systemic resistance (ISR) (Van Loon *et al.*, 1998). In contrast to pathogen-induced SAR, which is regulated by SA, rhizobacteria-mediated ISR is controlled by a signaling pathway in which ET and JA play key roles (Pieterse *et al.*, 1998). In the past decade, the model plant species *Arabidopsis thaliana* was explored to study the molecular basis of rhizobacteria-mediated ISR (Pieterse *et al.*, 2002). Here we review the 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 above-ground foliar tissues.

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2. Rhizobacteria-Induced Systemic Resistance (ISR)

To study the signal transduction pathway of ISR, an Arabidopsis-based model system was developed. In this model system, the nonpathogenic rhizobacterial strain *Pseudomonas fluorescens* WCS417r is used as the inducing agent (Pieterse *et al.*, 1996). Colonization of Arabidopsis roots by ISR-inducing WCS417r bacteria protects the plants against different types of pathogens, including the bacterial pathogens *Pseudomonas syringae* pv. *tomato* (*Pst*) DC3000, *Xanthomonas campestris* pv. *armoraciae*, and *Erwinia carotovora* pv. *carotovora*, the fungal root pathogen *Fusarium oxysporum* f.sp. *raphani*, the fungal leaf pathogens *Alternaria brassicicola* and *Botrytis cinerea*, and the oomycete pathogen *Hyaloperonospora parasitica* (Pieterse *et al.*, 1996; Ton *et al.*, 2002a).

Research on the molecular mechanism of ISR was initially focused on the role of pathogenesis-related (PR)-proteins, as the accumulation of these proteins was considered to be strictly correlated with induced disease resistance (Van Loon *et al.*, 2006). However, Arabidopsis plants expressing WCS417r-mediated ISR showed enhanced resistance against *F. oxysporum* 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). 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 expressed normally in SA-nonaccumulating Arabidopsis NahG plants (Pieterse *et al.*, 1996), and in the SA biosynthesis mutants *eds5/sid1* and *sid2* (Pieterse *et al.*, 2002). This led to the conclusion that WCS417r-mediated ISR is a SA-independent resistance mechanism, and that WCS417r-mediated ISR and pathogen-induced SAR are regulated by distinct signaling pathways.

3. Genetic Dissection of the ISR Pathway in Arabidopsis

Since SA was not involved in WCS417r-elicited ISR, the Arabidopsis JA-response mutants *jar1*, *coi1*, and *eds8*, and the ET-response mutant *etr1* were tested for their ability to express ISR. None of these mutants were unable to mount resistance against *Pst* DC3000 after colonization of the roots by WCS417r (Pieterse *et al.*, 1998; Ton *et al.*, 2002b; unpublished data), indicating that ISR requires responsiveness to both JA and ET (Fig. 1). To further elucidate the role of ET in the ISR signaling pathway, a large set of well-characterized ET-signaling mutants was analyzed. None of these mutants showed an ISR response against *Pst* DC3000 after colonization of the roots by WCS417r (Knoester *et al.*, 1999). These results confirmed that

an intact ET-signaling pathway is required for the establishment of ISR. Particularly interesting was the analysis of the *eir1* mutant, which is ET-insensitive in the roots, but not in the shoot. This *eir1* mutant was incapable of showing ISR after root colonization by WCS417r. In contrast, after leaf infiltration with WCS417r it did show ISR, indicating that responsiveness to ET is required at the site of rhizobacterial induction (Knoester *et al.*, 1999). The observation that the ET-responsive Arabidopsis *AtTLP1* gene, encoding a thaumatin-like protein, is activated in the roots upon colonization by WCS417r, confirms that ET signaling is initiated in the roots by ISR-inducing rhizobacteria (Léon-Kloosterziel *et al.*, 2005).

Further evidence for the involvement of the ET-response pathway came from the identification of the Arabidopsis *ISR1* locus (Ton *et al.*, 1999). Genetic analysis of the progeny of a cross between the WCS417r-responsive ecotype Col-0 and the ISR-impaired ecotype RLD1 revealed a single locus, designated *ISR1*, to be important in the expression of ISR against several different pathogens (Ton *et al.*, 2002c). Accessions with the recessive *isr1* allele have reduced sensitivity to ET and enhanced susceptibility to *Pst* DC3000 (Ton *et al.*, 2001). These results strongly indicate that the Arabidopsis *ISR1* locus encodes a novel component in the ET-signal transduction pathway that is important for both basal resistance and ISR in Arabidopsis.

4. Dual Role for NPR1 in SAR and ISR

To investigate a possible involvement of the SAR regulatory protein NPR1 in ISR signaling, the *Arabidopsis npr1* mutant was tested in the ISR bioassay. Surprisingly, the *npr1* mutant was incapable of showing WCS417r-mediated ISR (Pieterse *et al.*, 1998; Van Wees *et al.*, 2000) (Fig. 1). This result clearly showed that WCS417r-mediated ISR, like SA-dependent SAR, is an NPR1-dependent defense response. Further analysis of the ISR signal-transduction pathway revealed that NPR1 acts downstream of the JA- and ET-dependent steps (Pieterse *et al.*, 1998). Because SAR is associated with NPR1-dependent *PR*-gene expression, and ISR is not, the action of NPR1 in ISR must be different from that in SAR. These different activities are not mutually exclusive because simultaneous activation of ISR and SAR can lead to an enhanced defensive activity compared to that observed with either type of induced resistance alone (Van Wees *et al.*, 2000). These results suggest that the NPR1 protein is important in regulating and intertwining different hormone-dependent defense pathways.

5. ISR Is Associated with Priming for Enhanced Defense

In Arabidopsis, both JA and ET activate specific sets of defense-related genes (Schenk *et al.*, 2000) but, when applied exogenously, each of both can induce resistance (Pieterse *et al.*, 1998; Van Wees *et al.*, 1999). To investigate how far ISR is associated with these changes in JA/ET-responsive gene expression, Van Wees *et al.* (1999) monitored the expression of a set of well-characterized JA- and/or ET-responsive, defense-related genes in Arabidopsis plants expressing WCS417r-mediated ISR. None of these genes was up-regulated in induced plants, neither locally in the roots, nor systemically in the leaves. This suggested that the resistance attained was not associated with major increases in the levels of either JA or ET. Indeed, analysis of JA and ET levels in leaves of ISR-expressing plants revealed no changes in the production of these signal molecules (Pieterse *et al.*, 2000; Hase *et al.*, 2003). Therefore, it had to be assumed that the JA and ET dependency of ISR is based on an enhanced sensitivity to these hormones, rather than on an increase in their production.

To identify ISR-related genes, the transcriptional response of over 8000 Arabidopsis genes was monitored during WCS417r-mediated ISR (Verhagen *et al.*, 2004). However, systemically in the leaves, none of the ~8000 genes tested showed a consistent change in expression in response to effective colonization of the roots by WCS417r, indicating that the onset of ISR in the leaves is not associated with detectable changes in gene expression. However, after challenge inoculation of WCS417r-induced plants with *Pst* DC3000, 81 genes showed an augmented expression pattern in ISR-expressing leaves compared to inoculated control leaves, suggesting that ISR-expressing plants are primed to respond faster and/or more strongly upon pathogen attack. The majority of the primed genes were predicted to be regulated by JA and/or ET signaling, confirming earlier findings that colonization of the roots by WCS417r primed Arabidopsis plants for augmented expression of the JA- and/or ET-responsive genes *AtVSP2*, *PDF1.2* and *HEL* (Van Wees *et al.*, 1999; Hase *et al.*, 2003).

Priming is a phenomenon that is associated with different types of induced resistance (Conrath *et al.*, 2002; Conrath *et al.*, 2006). It provides the plant with an enhanced capacity for rapid and effective activation of cellular defense responses once a pathogen is contacted, and it allows the plant to react more effectively to any invader encountered by boosting the defenses that are activated in the host. This mechanism could also explain the broad-spectrum action of induced resistance. Priming for defense may combine advantages of enhanced disease protection with low metabolic costs. Recently, Van Hulten *et al.* (2006) examined the costs and benefits of priming in comparison to activated defense in Arabidopsis. The study

revealed that the benefits of priming-mediated resistance outweigh the costs under conditions of pathogen pressure, suggesting an evolutionary advantage of this mechanism of induced resistance over constitutive activation of defense responses. Figure 1 provides a schematic representation of the ISR signaling pathway in Arabidopsis.

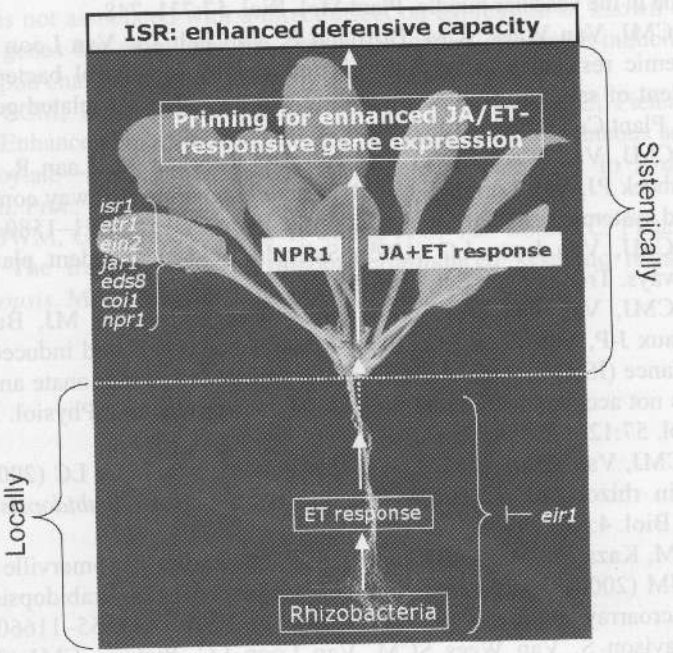

Fig. 1. Schematic representation of the *Pseudomonas fluorescens* WCS417r-mediated ISR signaling pathway in Arabidopsis.

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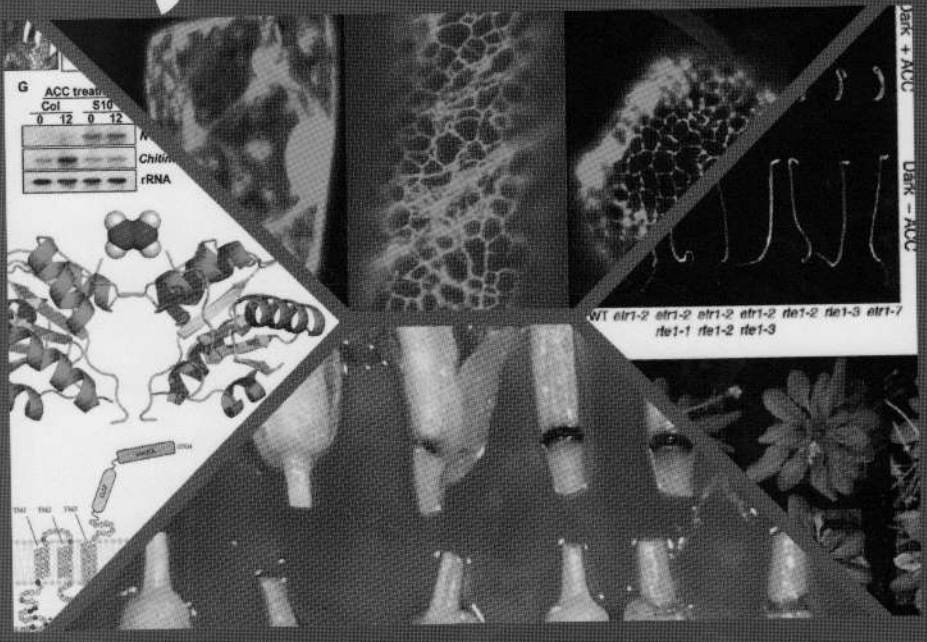

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