46. Signaling during rhizobacteria-induced systemic resistance in *Arabidopsis*

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Abstract

Arabidopsis plants of which the roots are colonized by specific strains of non-pathogenic fluorescent *Pseudomonas* bacteria develop an induced systemic resistance (ISR) that, unlike pathogen-induced systemic acquired resistance (SAR), is independent of salicylic acid (SA) but requires sensitivity to jasmonic acid (JA) and ethylene (ET). Various *Pseudomonas* spp. strains induce broad-spectrum disease resistance in plants in a bacterial strain/plant species-specific manner. Different bacterial determinants appear to be involved in triggering ISR, e.g. lipopolysaccharides (LPS), siderophores, flagella and antibiotics. Combining ISR and SAR increased resistance against pathogens that are resisted by both JA/ET- and SA-dependent defenses, but not against pathogens that are affected exclusively by either ISR or SAR. Unlike SAR, the onset of ISR is not associated with major changes in gene expression, but upon challenge inoculation ISR-expressing plants are primed to express certain sets of genes more quickly and/or at higher levels.

Introduction

Rhizobacteria-induced systemic resistance (ISR) is a type of induced resistance that is triggered by specific strains of non-pathogenic, root-colonizing bacteria, notably *Pseudomonas* spp. [19]. As a result of microbial stimulation, the capacity of the plant to defend itself against subsequent pathogenic attack is increased. This enhanced defensive capacity is expressed systemically, i.e. no contact between the inducing bacteria and the challenging pathogen is required. ISR has been shown to be active against pathogenic fungi, bacteria and viruses and, once induced, it can be maintained for prolonged periods. ISR has been demonstrated in *Arabidopsis*, bean, carnation, cucumber, radish, tobacco and tomato under greenhouse conditions and, in some cases, also in the field. Thus, ISR offers an attractive means to combat plant diseases in a natural, environmentally friendly way.

By making use of the power of *Arabidopsis* genetics, we developed a model system to study the induction, signaling, and expression of ISR in *A. thaliana* Col-0, using mainly *P. fluorescens* strain WCS417 as the inducer and the causal agent of bacterial speck, *P. syringae* pv. *tomato* DC3000 (*Pst*), as the challenging pathogen [13]. By testing several *Arabidopsis* accessions, as well as mutants and transgenics impaired in critical steps of disease resistance signaling, we established that WCS417 induces resistance by a signaling pathway that differs from that of pathogen-induced systemic acquired resistance (SAR) [15]. Whereas development of SAR requires salicylic acid (SA) as a signal, and is associated with the accumulation of pathogenesis-related proteins (PRs) with potential antimicrobial activities, ISR requires sensitivity to jasmonic acid (JA) and ethylene (ET), and is not associated with the activation of *PR* genes. Nevertheless, both SAR and ISR pathway require the adapter protein NPR1 (non-expresser of PRs), even though in ISR no PR expression is apparent [14].

Recently, in tomato ISR against late blight, induced by either *Bacillus pumilis* SE34 or *P. fluorescens* 89B61, was shown to be independent of SA, but dependent on JA and ET signaling [25].

Results and Discussion

Bacterial strain/plant species specificity in the induction of ISR

Of two further well-characterized rhizobacterial strains, *P. putida* WCS358 triggered ISR in *Arabidopsis* similarly to WCS417, whereas *P. fluorescens* WCS374 did not [22]. All three strains colonized *Arabidopsis* roots to similar levels, ruling out the possibility that WCS374 was unable to elicit ISR because of poor root colonization. WCS374 did induce resistance in radish, as did WCS417, but WCS358 did not [9]. WCS417 was likewise able to trigger ISR in carnation [20] and tomato [6], whereas WCS358 tested on carnation, did not. These results demonstrate that the three *Pseudomonas* strains are differentially active in triggering ISR in these three plant species, indicating specific recognition between bacteria and plants at the root surface.

In order to study which bacterial factors are involved in the elicitation of ISR by WCS358 in *Arabidopsis*, selected mutants as well as purified components were tested [2]. In this way it was established that several bacterial determinants contribute to the ability of the two strains to elicit ISR. For instance, wild type (wt) WCS358 reduced bacterial speck disease on average by about 45%. A mutant lacking the O-antigenic side chain of the outer membrane lipopolysaccharide (LPS^-) induced resistance to a similar level. However, washed cell wall preparations from the wild type (wt) induced resistance to about the same extent, implicating the LPS in resistance induction. Similar results were obtained with, on the one hand, a mutant lacking the pseudobactin siderophore and, on the other hand, the purified pseudobactin. Moreover, a double mutant lacking both traits was still effective in inducing resistance, indicating that a third determinant must be involved. At least purified flagella proved also active in inducing resistance.

The situation for WCS417 is similar for the elicitation of ISR by LPS. A LPS^- mutant still induced resistance and cell walls of the wt but not those of the LPS^- mutant induced resistance, suggesting multiple inducing determinants also in WCS417 [22]. However, the pseudobactin siderophore did not seem to play a role. Whereas elicitation of ISR by WCS417 in carnation appeared to be solely attributable to the LPS [21], in radish in addition to the LPS a Fe-regulated compound, different from the pseudobactin siderophore, could be implicated [10]. The nature of this compound is not known.

When comparing established bacterial determinants from other well-characterized resistance-inducing *Pseudomonas* spp., the siderophore was also found to play a role in the induction of resistance in tobacco by *P. fluorescens* strain CHA0 [12]. Interestingly, bacterially-produced SA was implicated as the determinant responsible for the elicitation of ISR by *P. aeruginosa* strain 7NSK2 in bean against *Botrytis cinerea* [4] and in tobacco against tobacco mosaic virus [5]. In a recent study in tomato, however, a combination of the pyochelin siderophore (containing a SA moiety) and the antibiotic pyocyanin appears to be required for ISR by 7NSK2 against *B. cinerea* [1], indicating again that different plant species perceive different bacterial determinants.

ISR and SAR have complementary protective actions

The effectiveness of ISR against different types of pathogens was compared with that afforded by SAR. *Arabidopsis* plants were grown in soil containing WCS417 and challenged when 5 weeks old, by dipping the leaves in a suspension of virulent *Pst* bacteria for induction of ISR. For induction of SAR, plants were grown in autoclaved soil and 3 days before challenge with virulent *Pst* pressure-infiltrated in the first pair of true leaves with a suspension of avirulent *Pst*. For determining the effect of a combination of ISR and SAR, both treatments were applied to the same plants. Induction of SAR proved to be slightly, but reproducibly, more effective than ISR [16, 24]. When instead of *Pst*, *Xanthomonas campestris* pv. *armoracia* (*Xca*) was used as the challenging pathogen, both treatments were equally effective. ISR conferred only weak protection against the downy mildew oomycete *Peronospora parasitica*, whereas activation of SAR resulted in a high level of protection against this pathogen [17]. Against the fungus *Fusarium oxysporum* f.sp. *raphani*, ISR and SAR were about equally effective [13]. However, elicitation of ISR resulted in a significant level of protection against *Alternaria brassicicola*, whereas SAR was ineffective. Converse-

ly, SAR was effective against turnip crinckle virus (TCV), whereas ISR was not at all [17]. This spectrum of activity fits with the known signaling pathways involved in basal resistance of *Arabidopsis* against these various pathogens, as evidenced by the enhanced disease susceptibility of mutants impaired in JA, ET or SA signaling: SAR was effective against pathogens that in non-induced plants are resisted through SA-dependent basal resistance responses – notably biotrophs – while ISR protected against pathogens that in non-induced plants are restricted through JA/ET-dependent basal resistance responses – particularly necrotrophs. However, there is considerable overlap, as evidenced by the dual effectiveness of SAR and ISR against most of the pathogens investigated. Nevertheless, these results strongly suggest that WCS417r-mediated ISR involves an enhancement of JA- and ET-dependent basal resistance, whereas SAR constitutes an enhancement of SA-dependent basal resistance [17], in accordance with the earlier notion that induced disease resistance is an enhancement of genetically determined basal resistance by which extant defense mechanisms are expressed earlier and to higher levels [18].

Because ISR and SAR appear to have complementary effects in counteracting diseases caused by different pathogens, it became of interest to study whether combinations of both types of induced resistance would enhance and broaden the spectrum of effectiveness. As both ISR and SAR signaling require the regulatory protein NPR1, such experiments were also relevant to assess in how far NPR1 plays a limiting role in the acquisition of induced resistance. Such a limitation could be envisaged because overexpression of the allelic *Nim1* gene has been shown to enhance disease resistance [7].

Induction of both ISR and SAR resulted in a more than doubling of the protection against *Pst* in challenged leaf tissues compared to induction of ISR alone, and 40% more protection compared to SAR alone [24]. No enhanced level of protection was evident in JA-insensitive *jar1* or in ET-insensitive *etr1* mutant plants, in which ISR but not SAR was impaired, or in NahG transformants that were blocked in SAR but still able to fully express ISR. Thus, the ISR and the SAR pathways appeared to function independently and additively. Leaves expressing both types of induced resistance did not show elevated levels of *Npr1* transcripts, suggesting that the additive effect of ISR and SAR on induced protection relies neither on enhanced levels, nor on a limiting amount of NPR1 protein. Because ISR and SAR are equally effective against *Xca*, dually induced plants were likewise tested against *Xca*. A comparable additive effect of both types of induced resistance was found against this pathogen also. In contrast, no such additive effect was apparent in plants challenged with *A. brassicicola*, that is resisted only through ISR, or inoculated with TCV, that is reduced only through SAR. Nevertheless, plants expressing both ISR and SAR expressed the enhanced resistance against *A. brassicicola* afforded by ISR, as well as the protection against TCV resulting from SAR.

Priming of defense responses in induced plants

Whereas in plants with SAR, genes encoding PRs are expressed to substantial levels upon induction, no changes in gene expression were evident in either roots or leaves of plants in which effective ISR had been induced. Neither were genes coding for several further known defense-related genes found to be activated upon ISR induction [23]. To identify ISR-related genes, expression profiles of over 8000 genes were analyzed using Affymetrix Gene Chip *Arabidopsis* Genome Arrays. Arrays were hybridized with probes derived from RNA isolated from control and ISR-expressing plants that were extracted at different times after induction and before and after challenge inoculation with *Pst*. Seedling roots treated with WCS417 displayed transient alterations in the expression of about 100 genes. It remains to be investigated in how far any of these changes are related to the induction of ISR because colonization of roots by rhizobacteria is likely to also affect the expression of plant genes related to growth and metabolism [8]. In the leaves of the same plants, expression of none of the genes was altered, supporting our previous results that the onset of ISR is not associated with major changes in gene expression.

Upon infection with a pathogen, *Arabidopsis* reacts by expressing a large set of defense-related genes [11]. Of these, after challenge inoculation of leaves of ISR-expressing plants with *Pst*, several showed a potentiated expression, suggesting that these genes were primed to be expressed more quickly and/or to higher levels upon pathogen attack. Among these genes were several JA-responsive ones, such as *AtVsp* [23], indicating that ISR involves potentiation of the response to the defense signaling compound JA. Moreover, additional genes were activated in challenged ISR-expressing plants compared to challenged, non-induced plants. Because no significant alterations were apparent in the leaves before challenge inoculation, ISR appears to correspond to a primed state in which no major investment in new synthesis is made, but responsiveness of the plant to pathogen attack is enhanced [3]. This priming of defense-related activities will help the plant to combat invading pathogens more effectively, resulting in enhanced protection against a broad spectrum of pathogens.

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