

Genetic analysis of induced systemic resistance in *Arabidopsis thaliana*: association between induced and basal resistance

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Abstract

Selected nonpathogenic rhizobacteria are able to elicit induced systemic resistance (ISR) in plants. Different ecotypes of *Arabidopsis thaliana* were screened for expression of ISR against infection by *Pseudomonas syringae* pv. *tomato*, after treatment of the roots with the nonpathogenic *P. fluorescens* strain WCS417r. From the seven ecotypes tested, two ecotypes (RLD and Ws-0) did not develop ISR after treatment with *P. fluorescens* strain WCS417r. This ISR-nonresponsive phenotype was correlated with a remarkably low level of basal resistance, suggesting that the ability to express ISR is dependent on a threshold level of basal resistance. Subsequently, a cross was made between an ISR-responsive (Col-0) and an ISR-nonresponsive ecotype (RLD). F₁ hybrids were fully capable of expressing ISR and exhibited a relatively high level of basal resistance, indicating that the potential to express ISR and basal resistance are both inherited as dominant traits. Analysis of F₂ plants revealed that both traits segregated in a 3 : 1 fashion in the same set of plants, indicating that the potential to express ISR and basal resistance are monogenically determined and presumably linked.

Introduction

Induced disease resistance is the phenomenon that plants develop an enhanced defensive capacity against a broad spectrum of pathogens upon appropriate stimulation. This resistance response is expressed systemically throughout the plant and is effective against a broad spectrum of pathogens (Hammerschmidt and Kuc 1995). In general, there are two types of induced disease resistance: pathogen-induced systemic acquired resistance (SAR) and rhizobacteria-mediated induced systemic resistance (ISR). SAR is a defense mechanism that is dependent on salicylic acid (SA) and is associated with the accumulation of pathogenesis-related (PR) proteins (Ryals et al. 1996). ISR is a resistance response that is triggered after root treatment with selected strains of nonpathogenic rhizobacteria. Using *Arabidopsis* as a model, it was shown that ISR follows a signalling pathway that, unlike SAR, is independent of SA and PR-gene activation (Pieterse et al. 1996, Van Wees et al. 1997). Apparently, rhizobacteria-mediated ISR and pathogen-induced SAR are controlled by different signalling pathways. However, upon challenge inoculation, SAR and ISR are both expressed as a reduction in disease incidence or severity, suggestive of a similar defensive mechanism becoming activated. After infection by a pathogen, SAR-induced plants are characterized by a rapid and intense activation of a broad spectrum of defense mechanisms. So far, cell wall lignification (Hammerschmidt and Kuc 1982), increased peroxidase activity (Simons and Ross 1970, Van Loon 1976, Ye et al. 1990), accumulation of hydroxyproline-rich proteins (Hammerschmidt et al. 1984), callose deposition (Ye et al. 1991), and accumulation of antifungal PR-proteins (Van Loon 1985) have been reported as possible mechanisms contributing to the enhanced resistance state of SAR. In contrast, not much is known about the

defense mechanisms that are activated during rhizobacteria-mediated ISR.

The main objective of this study was to elucidate the genetic basis of rhizobacteria-mediated ISR and to investigate the relationship between ISR and basal resistance in *Arabidopsis*. Based on the observation of Van Wees et al. (1997) that the *Arabidopsis* ecotype RLD fails to express ISR after treatment with *P. fluorescens* strain WCS417r, we started a classic genetic approach. Several *Arabidopsis* ecotypes were screened for their potential to express WCS417r-mediated ISR against the leaf pathogen *P. syringae* pv. *tomato*. Subsequently, crosses were made between WCS417r-responsive and WCS417r-nonresponsive ecotypes. In this study, we demonstrate that the WCS417r-nonresponsive phenotype of two *Arabidopsis* ecotypes correlates with a remarkably low level of basal resistance against *P. syringae* pv. *tomato*. Furthermore, we provide evidence that the potential to express WCS417r-mediated ISR and basal resistance against *P. syringae* pv. *tomato* are both inherited as a single dominant trait.

Materials and Methods

The rifampicin-resistant *P. fluorescens* strain WCS417r (Van Peer et al. 1991), was grown on King's medium B agar plates (King et al. 1954) for 24 h at 28 °C. The virulent bacterial leaf pathogen *P. syringae* pv. *tomato* strain DC3000 and the avirulent strain *P. syringae* pv. *tomato* DC3000 (*avrRpt2*) (Whalen et al. 1991) were cultured overnight in liquid King's medium B at 28 °C. The bacterial cells were collected and resuspended in 10 mM MgSO₄. Seedlings of *A. thaliana* ecotypes Col-0, Ler, Cvi, Shah, Kas, RLD, and Ws-0, and F₁ plants of the Col-0 x RLD cross were grown in quartz sand for two weeks. Subsequently they were transferred to pots (60 ml), containing a sand/potting soil mixture that had been autoclaved twice before application of WCS417r or 10 mM MgSO₄. Plants were cultivated in a growth chamber with a 9-h day (200 mE/m².sec at 24 °C) and 15-h night (20 °C) cycle and 65% relative humidity. Plants were watered on alternate days and once a week supplied with nutrient solution.

The *Arabidopsis*-*P. syringae* bioassay. Prior to transfer of the *Arabidopsis* seedlings to the pots, a suspension of ISR-inducing WCS417r bacteria (10⁹ CFU/ml) was mixed thoroughly through the sterile sand/potting soil mixture to a final density of 5 x 10⁷ CFU/g. Control soil was supplemented with an equal volume of sterile 10 mM MgSO₄. SAR induction was performed 4 days before challenge inoculation by pressure-infiltrating two lower leaves with a suspension of *P. syringae* pv. *tomato* (*avrRpt2*) at 10⁷ CFU/ml in 10 mM MgSO₄. One day before challenge inoculation, the plants were placed at 100% relative humidity. Five-week-old plants were inoculated by dipping the leaves in a *P. syringae* pv. *tomato* suspension containing 2.5 x 10⁷ CFU/ml in 10 mM MgSO₄, 0.015% (v/v) Silwet L-77 (Van Meeuwen Chemicals BV, Weesp, The Netherlands). Four or five days after challenge inoculation, the proportion of leaves with symptoms was determined per plant (n = 20-25). Data were statistically analyzed using Student's t-test or an analysis of variance followed by the Fisher's LSD test.

For quantification of basal resistance, leaves of 5-week-old plants were inoculated by pressure infiltrating a suspension of *P. syringae* pv. *tomato* at 5 x 10⁵ CFU/ml in 10 mM MgSO₄. Two days after inoculation, five leaf samples per ecotype were collected, weighed, and homogenized in 10 mM MgSO₄. Subsequently, dilutions were plated onto selective King's medium B agar supplemented with 50 mg/L rifampicin and 100 mg/L cycloheximide. After incubation for 48 h at 28 °C, the number of rifampicin-resistant CFU per g of infected leaf tissue was determined.

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To investigate whether the potential to express ISR and basal resistance are monogenic or multigenic traits, F₂ plants of the Col-0 x RLD hybrids were analysed. Approximately 25% of the F₂ plants (11 out of 47) were nonresponsive to WCS417r-treatment and exhibited a disease severity level comparable with RLD plants. Approximately 75% of the F₂ plants (36 out of 47) were responsive to WCS417r treatment and exhibited a disease severity level comparable with WCS417r-treated Col-0 plants. The observation that basal resistance and the potential to express ISR follow a 3 : 1 segregation in the same set of plants, suggests that both traits are monogenically determined and linked.

Table 2. Quantification of induced resistance and basal resistance against *P. syringae* pv. *tomato* in Col-0, RLD and F₁ hybrids of the Col-0 x RLD cross^Y.

| | Control treatment ^Z | WCS417r treatment ^Z |
|----------------|--------------------------------|--------------------------------|
| Col-0 | 100a | 36b |
| RLD | 184a | 210a |
| F ₁ | 92a | 46b |

^Y The bioassays for the quantification of induced resistance was performed as described in Materials and Methods. The level of basal resistance can be deduced from the disease severity observed in control-treated plants.

^Z Numbers are the proportion of leaves with symptoms relative to the control treatment of Col-0 (100%) at 4 days after challenge inoculation. Different letters in the same row indicate statistically significant differences compared to the control treatment (Student's t-test, $P=0.05$).

Discussion

Rhizobacteria-mediated ISR and pathogen-induced SAR are two plant resistance responses that are controlled by different signalling pathways (Pieterse et al. 1996, Van Wees et al. 1997). The observation that pathogen-induced SAR can be expressed in the *Arabidopsis* ecotypes RLD and Ws-0, whereas WCS417r-mediated ISR is not (Table 1), provides additional evidence that ISR and SAR are different plant responses. However, the cause of the WCS417r-nonresponsive phenotype of RLD and Ws-0 remains to be clarified. RLD and Ws-0 might be affected in the recognition of the ISR-inducing rhizobacteria, or alternatively, be disturbed in the signalling pathway controlling ISR. Another explanation could be that expression of ISR in RLD and Ws-0 is dependent on the level of basal resistance. The observation that RLD and Ws-0 have a remarkably low level of basal resistance against *P. syringae* pv. *tomato* (Table 1), suggests that the potential of a plant to express ISR requires resistance mechanisms to be sufficiently active against the challenging pathogen. We demonstrated that basal resistance and the potential to express ISR are both inherited as a dominant trait in F₁ plants of the Col-0 x RLD cross (Table 2). Furthermore, we showed that basal resistance and potential to express ISR both follow a 3 : 1 segregation in the same set of F₂ plants, indicating that both traits are monogenically inherited and presumably linked. The results support the notion that ISR is an enhancement of extant resistance (Van Loon 1983). RLD and Ws-0 appear to lack the ability to express ISR against *P. syringae* pv. *tomato* because they possess only a low level of basal resistance against this pathogen.

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