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# Heritability of rhizobacteria-mediated induced systemic resistance and basal resistance in Arabidopsis

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**Abstract**

Selected strains of non-pathogenic rhizobacteria have the ability to trigger an induced systemic resistance (ISR) response in plants. In Arabidopsis, rhizobacteria-mediated ISR has been extensively studied, using *Pseudomonas fluorescens* WCS417r as the inducing agent and *P. syringae* pv. *tomato* DC3000 (*Pst*) as the challenging pathogen. To investigate how far expression of ISR depends on the level of basal resistance, 10 different Arabidopsis ecotypes were screened for their potential to express WCS417r-mediated ISR and basal resistance against *Pst*. Two Arabidopsis ecotypes, RLD and Wassilewskija (Ws), were found to be blocked in their ability to express ISR. This ISR-noninducible phenotype correlated with a relatively low level of basal resistance against *Pst*. Genetic analysis of crosses between the ISR-inducible ecotypes Columbia (Col) and Landsberg *erecta* (L*er*), on the one hand, and the non-inducible ecotypes RLD and Ws, on the other hand, revealed that ISR inducibility and basal resistance against *Pst* were inherited as monogenic dominant traits that are genetically linked. Neither ISR inducibility, nor basal resistance against *Pst* was complemented in the $F_1$ progeny of a cross between RLD and Ws, indicating that both ecotypes are affected in the same locus. This locus, designated *ISR1*, was mapped between markers *Ein3* and *GL1* on chromosome III. Interestingly, ecotypes RLD and Ws also failed to express ISR against the oomycetous pathogen *Peronospora parasitica*, but they were not affected in their level of basal resistance against this pathogen. Thus, the *ISR1* locus controls the expression of ISR against different pathogens but basal resistance only against *Pst* and not against *P. parasitica*. Like ecotypes RLD and Ws, ethylene-insensitive mutants showed the *isr1* phenotype in that they were unable to express WCS417r-mediated ISR and show enhanced susceptibility to *Pst* infection. Analysis of ethylene responsiveness of RLD and Ws revealed that both ecotypes exhibit reduced sensitivity to ethylene. Therefore, it is proposed that the Arabidopsis *ISR1* locus encodes a component of the ethylene-response pathway that plays an important role in ethylene-dependent resistance mechanisms.

*Abbreviations:* CAPS – cleaved amplified polymorphic sequence; ISR – induced systemic resistance; JA – jasmonic acid; PRs – pathogenesis-related proteins; *Pst* DC3000 – *Pseudomonas syringae* pv. *tomato D*C3000; SA – salicylic acid; SAR – systemic acquired resistance.

## Systemically induced disease resistance

When appropriately stimulated, plants systemically enhance their defensive capacity against pathogen attack. This induced resistance is generally characteriseds by a restriction of pathogen growth and a reduction in disease severity (Hammerschmidt, 1999). The development of a broad-spectrum, systemic acquired

resistance (SAR) after primary pathogen infection has been studied extensively (Ryals et al., 1996). This pathogen-induced SAR is characterised by an early increase in endogenously synthesised salicylic acid (SA) (Malamy et al., 1990; Métraux et al., 1990). SA is an essential signalling compound in the SAR signalling pathway, because transgenic plants that are unable to accumulate SA are incapable of developing SAR (Gaffney et al., 1993). Furthermore, SAR is associated with the systemic activation of so-called SAR genes. These include genes that encode pathogenesis-related (PR) proteins (Ward et al., 1991), some of which have *in vivo* antifungal activity and are therefore thought to contribute to the resistance state of SAR (Ryals et al., 1996; Van Loon, 1997).

Selected strains of non-pathogenic, root-colonising rhizobacteria have been shown to be capable of inducing disease resistance as well. This phenomenon is commonly referred to as rhizobacteria-mediated induced systemic resistance (ISR; Van Loon et al., 1998). Rhizobacteria are present in large numbers on the root surface, where plant exudates and lysates provide nutrients (Lynch and Whipps, 1991). Besides inducing resistance, rhizobacterial strains have been reported to also directly antagonise soil-borne pathogens and to stimulate plant growth (Bakker et al., 1991; Wei et al., 1996; Pieterse and Van Loon, 1999). Since the discovery of the resistance-inducing capacities of rhizobacteria in the early 1990s, ISR has been demonstrated in different plant species against a broad spectrum of pathogens (Van Loon et al., 1998). The mechanisms by which rhizobacteria induce resistance vary. Some rhizobacterial strains trigger the SA-dependent resistance pathway by producing SA at the root surface (De Meyer and Hofte, 1997; Maurhofer et al., 1998; De Meyer et al., 1999), whereas others activate a SA-independent pathway (Pieterse et al., 1996; Press et al., 1997).

*Arabidopsis thaliana* has proved to be an attractive model species for the elucidation of the molecular mechanisms underlying rhizobacteria-mediated ISR. Using *Pseudomonas fluorescens* WCS417r as the inducing agent, it was demonstrated that ISR occurred without concomitant expression of SAR genes (Pieterse et al., 1996; Van Wees et al., 1999). Moreover, the ISR signalling pathway functioned independently of SA, but required an intact response to the plant hormones jasmonate (JA) and ethylene (Pieterse et al., 1998; Knoester et al., 1999). Further investigations revealed that induction of ISR did not result in enhanced production of JA and ethylene (Pieterse et al., 2000), nor in the expression of ethylene- and JA-responsive genes (Van Wees et al., 1999). These observations suggest that ISR is based on a sensitisation of the tissue for these hormones, rather than on an increase in their production. Despite the differences between WCS417r-mediated ISR and pathogen-induced SAR, both resistance responses were found to be blocked in the *npr1-1* mutant, indicating that both SAR and ISR are controlled by the regulatory protein NPR1 (Cao et al., 1994; Pieterse et al., 1998). Downstream of NPR1 both pathways diverge, indicating that NPR1 differentially regulates defence responses depending on the pathway that is activated upstream of it (Pieterse et al., 1998). Interestingly, simultaneous activation of both the JA/ethylene-dependent ISR pathway and the SA-dependent SAR pathway resulted in an enhanced level of protection against *Pseudomonas syringae* pv. *tomato* DC3000 (*Pst*) (Van Wees et al., 2000), indicating that the effects of ISR and SAR are additive.

## Naturally occurring variation in ISR inducibility and basal resistance against *Pst*

The capacity to express *P. fluorescens* WCS417r-mediated ISR was found to be dependent on the plant genotype. For instance, ecotypes Columbia (Col) and Landsberg *erecta* (L*er*) were responsive to induction of ISR by WCS417r, whereas ecotype RLD was not (Van Wees et al., 1997; Ton et al., 1999). In carnation, cultivar specificity with regard to expression of WCS417r-mediated ISR has also been reported. ISR induced by WCS417r against fusarium wilt, caused by *Fusarium oxysporum* f.sp. *dianthi*, was clearly expressed in the moderately resistant cultivar Pallas, but less strongly and consistently in the susceptible cultivar Lena (Van Peer et al., 1991). This suggests that the level of basal resistance can influence the extent to which WCS417r-mediated ISR is expressed. Such observations fit the hypothesis that induced resistance constitutes an enhancement of extant defensive mechanisms (Van Loon, 1997). However, several situations have been reported in which a clear correlation between the capacity to express rhizobacteria-mediated ISR and the level of basal resistance against the challenging pathogen was absent. For instance, in cucumber two susceptible cultivars expressed ISR after treatment with *Serratia marcescens* 90-166, whereas a resistant cultivar did not (Liu et al., 1995). Moreover, both susceptible and resistant cultivars of radish were capable

of expressing *P. fluorescens* WCS374-mediated ISR against fusarium wilt to the same extent (Leeman et al., 1995).

To further elucidate the relationship between *P. fluorescens* WCS417r-mediated ISR and basal resistance against *Pst* in Arabidopsis, a genetic approach using the natural variation in ISR inducibility between ecotypes was persued. Out of the 10 ecotypes tested, RLD and Wassilewskija (Ws) failed to develop ISR after treatment of roots with WCS417r bacteria, whereas they were fully capable of expressing SAR after predisposal infection with avirulent *Pst*(*avrRpt2*) (Table 1; Ton et al., 1999). This ISR-noninducible phenotype of RLD and Ws could not be attributed to poor root colonisation by the ISR-inducing WCS417r bacteria, because both ecotypes allowed levels of root colonisation comparable to that of the ISR-inducible ecotypes (Ton et al., 1999). Remarkably, the ISR-noninducible phenotype of RLD and Ws correlated with a relatively low level of basal resistance against *Pst*. After challenge inoculation, disease symptoms in RLD and Ws were characterised by many large necrotic or water-soaked lesions, surrounded by extensive chlorosis, whereas disease on Col plants was more restricted and significantly less severe (Ton et al., 1999). Moreover, RLD and Ws allowed at least 5-fold higher levels of growth of *Pst* compared to the ISR-inducible ecotypes (Table 1; Ton et al., 1999). Apparently, RLD and Ws lack one or more genetic traits that are not only involved in the expression of ISR, but also contribute to basal resistance against *Pst*.

*Table 1*. Quantification of *P. fluorescens* WCS417r-mediated ISR, pathogen-induced SAR and basal resistance against *Pst* in different Arabidopsis ecotypes[a]

| Ecotype[a] | ISR (% induced protection)[a] | SAR (% induced protection)[a] | Basal resistance[b] |
|---|---|---|---|
| Col | 42.2* | 61.8* | 2.36 ± 0.18 |
| L*er* | 45.8* | 57.8* | 2.83 ± 0.08 |
| Cvi | 19.3* | 37.6* | 3.06 ± 0.17 |
| Sha | 32.3* | 43.1* | 2.71 ± 0.35 |
| Kas | 17.0* | 45.2* | 2.17 ± 0.23 |
| C24 | 25.7* | 38.7* | 2.12 ± 0.09 |
| Wei | 27.9* | 38.0* | 2.96 ± 0.22 |
| Ren | 41.5* | 54.9* | 2.77 ± 0.18 |
| RLD | −4.9 | 62.8* | 3.61 ± 0.09 |
| Ws | −5.3 | 42.8* | 3.71 ± 0.21 |

[a]ISR was induced by transferring 2-week-old seedlings to soil containing *P. fluorescens* WCS417r bacteria at $5 \times 10^7$ cfu $g^{-1}$. SAR was induced by pressure infiltrating a suspension of avirulent *Pst* (*Pst*(*avrRpt2*)) at $10^7$ cfu $ml^{-1}$ into two lower leaves 4 days prior to challenge inoculation. Five-week-old plants were challenge-inoculated by dipping the leaves in a bacterial suspension of *Pst* at $2.5 \times 10^7$ cfu $ml^{-1}$. Three to 5 days later, the plants were scored for disease symptoms. Induced protection is presented as the reduction in percentage of leaves with disease symptoms, relative to that of challenged control plants. Asterisks indicate statistically significant differences compared to non-induced control plants (Fisher's LSD test; $\alpha = 0.05$; $n = 20$–25). [b]Values presented are means (± SD) of the log of the proliferation of *Pst* over a 3-day time interval. Five-week-old plants were infected by pressure infiltrating a suspension of virulent *Pst* at $5 \times 10^5$ cfu $ml^{-1}$ into the leaves. Immediately after pressure infiltration and 3 days later, the number of *Pst* bacteria per gram of leaf fresh weight was determined.

## Identification of the *ISR1* locus controlling both WCS417r-mediated ISR and basal resistance against *Pst*

The naturally occurring variation in ISR inducibility and basal resistance against *Pst* enabled a genetic analysis to be performed by crossing the ISR-noninducible ecotypes RLD and Ws with the ISR-inducible ecotypes Col and L*er*. The resulting $F_1$ progenies were all capable of expressing ISR and showed comparable levels of growth of *Pst* in their leaves as the corresponding ISR-inducible parents (Table 2; Ton et al., 1999). These results demonstrated that ISR inducibility and basal resistance against *Pst* are both inherited as dominant traits. To investigate whether these traits are mono- or multigenic, the $F_2$ progeny of the RLD × Col cross was examined for segregation of ISR inducibility and basal resistance. Of the 98 $F_2$ plants tested, 28 plants were nonresponsive to WCS417r treatment and exhibited a level of disease severity comparable to that of RLD plants, whereas 70 plants were responsive to WCS417r treatment and showed disease severity similar to that in ISR-expressing Col plants. These data fit a statistically significant 3 : 1 segregation ($\chi^2 = 0.667$; $P = 0.414$) indicating that both ISR inducibility and basal resistance against *Pst* are monogenically inherited.

To further investigate the inheritance of ISR inducibility in relation to basal resistance, individual $F_2$ plants of the Col × RLD cross were selfed, resulting in 74 $F_3$ families. Subsequently, 16 non-induced $F_3$ plants of each family were challenge inoculated with *Pst*, whereupon the disease symptoms were monitored after 3, 4 and 5 days. Evaluation of symptoms revealed that 17 $F_3$ families were homozygous for the moderate

*Table 2*. Quantification of *P. fluorescens* WCS417r-mediated ISR and basal resistance against *Pst* in different Arabidopsis ecotypes and $F_1$ plants

| Genotype | ISR (% induced protection)[a] | Basal resistance[b] |
|---|---|---|
| Col | 35.7* | 2.36 ± 0.18 |
| Col × RLD | 49.9* | 2.24 ± 0.15 |
| Col × Ws | 50.0* | 2.35 ± 0.07 |
| L*er* | 36.0* | 2.83 ± 0.08 |
| L*er* × Ws | 29.0* | 2.80 ± 0.08 |
| RLD | −3.1 | 3.61 ± 0.09 |
| Ws | −0.5 | 3.71 ± 0.21 |
| Ws × RLD | − 0.1 | 3.51 ± 0.24 |

[a]ISR was induced by transferring 2-week-old seedlings to soil containing *P. fluorescens* WCS417r bacteria at $5 \times 10^7$ cfu $g^{-1}$. Five-week-old plants were challenge-inoculated by dipping the leaves in a bacterial suspension of *Pst* at $2.5 \times 10^7$ cfu $ml^{-1}$. Three days after challenge inoculation, the plants were scored for disease symptoms. Induced protection is presented as the reduction in percentage of leaves with disease symptoms, relative to that of challenged control plants. Asterisks indicate statistically significant differences compared to non-induced control plants (Student's *t* test $\alpha = 0.05$).

[b]Values presented are means (± SD) of the log of the proliferation of *Pst* over a 3-day time interval. Five-week-old plants were infected by pressure infiltrating a suspension of virulent *Pst* at $5 \times 10^5$ cfu $ml^{-1}$ into the leaves. Immediately after pressure infiltration and 3 days later, the number of *Pst* bacteria per gram of leaf fresh weight was determined.

Col-like disease symptoms, 40 $F_3$ families were heterozygous, showing both moderate and severe disease symptoms, and 17 $F_3$ families were homozygous for severe RLD-like disease symptoms. This segregation of disease severity in the $F_3$ families fits a Mendelian 1 : 2 : 1 segregation ($\chi^2 = 0.486$; $P = 0.784$), confirming the monogenic inheritance of basal resistance against *Pst*. Five RLD-like families and 5 Col-like families were tested for growth of *Pst* in the leaves and responsiveness to induction of ISR by WCS417r. The 5 $F_3$ families characterised by Col-like disease symptoms were fully capable of expressing WCS417r-mediated ISR and allowed relatively low levels of growth of *Pst*. In contrast, the 5 $F_3$ families characterised by RLD-like disease symptoms, did not develop ISR upon treatment with WCS417r and allowed relatively high levels of growth of *Pst*. This apparent co-segregation of ISR inducibility and relatively high basal resistance, on the one hand, and ISR noninducibility and relatively low basal resistance, on the other hand, demonstrates that both defence mechanisms are genetically linked. The corresponding locus was designated *ISR1* (Ton et al., 1999).

Using the homozygous RLD-like (*isr1/isr1*) and Col-like (*ISR1/ISR1*) $F_3$ families as genetic populations, cleaved amplified polymorphic sequence (CAPS) analysis was performed to locate the genetic map position of the *ISR1* locus. The *ISR1* locus cosegregated with the markers *B4* ($\chi^2 = 13.5$; $P = 0.001$), *Ein3* ($\chi^2 = 18.1$; $P < 0.001$), *GL1* ($\chi^2 = 18.0$; $P < 0.001$), and *BGl1* ($\chi^2 = 5.5$; $P = 0.064$) on chromosome III. Of the 32 segregants tested, 15 chromosomes were recombinant with *B4*, 13 with *Ein3*, 14 with *GL1*, and 23 with *BGL1*, yielding recombination frequencies of 23.4%, 21.9%, 20.3% and 35.9%, respectively. These recombination frequencies indicate that the *ISR1* locus is located on chromosome III between markers *Ein3* and *GL1*.

## Arabidopsis ecotypes RLD and Ws are both affected in the *ISR1* locus

Ecotype Ws showed the same phenotype as RLD, and upon crossing with Col or L*er*, the phenotype was likewise found to be recessive (Table 2). To investigate whether RLD and Ws are affected in the same trait, a cross between the two ecotypes was made and the progeny was analysed for both ISR inducibility and basal resistance against *Pst*. The resulting $F_1$ progeny behaved as their ISR-noninducible parents: they failed to develop WCS417r-mediated ISR and allowed relatively high levels of growth of *Pst* in their leaves, comparable to that of both parents (Table 2; Ton et al., 1999). These findings demonstrated that RLD and Ws were unable to complement each other for the ability to express ISR and basal resistance against *Pst*, indicating that both ecotypes are affected in the *ISR1* locus.

## The *ISR1* locus is not involved in basal resistance against *Peronospora parasitica*

The involvement of the *ISR1* locus in both ISR and basal resistance against *Pst* suggests that *ISR1* encodes a common component that is involved in both resistance responses. To investigate whether this locus similarly regulates ISR and basal resistance against a different pathogen, the resistance responses of ecotypes Col and Ws against the downy mildew-causing oomycetous leaf pathogen *P. parasitica* were studied.

*Table 3*. Quantification of *P. fluorescens* WCS417r-mediated ISR and basal resistance against *P. parasitica* in the Arabidopsis ecotypes Col (*ISR1/ISR1*) and Ws (*isr1/isr1*)

| Ecotype | Treatment[a] | Disease rating[b] (no. leaves per class) | | | | Total no. leaves | % leaves with spores | No. isolated sporesg$^{-1}$ FW[c] |
|---|---|---|---|---|---|---|---|---|
| | | 0 | 1 | 2 | 3 | | | |
| Col | Control | 80 | 45 | 66 | 43 | 234 | 66 | $10.8 \times 10^5$ |
| | WCS417r* | 128 | 12 | 33 | 27 | 200 | 36 | $1.5 \times 10^5$ |
| Ws | Control | 118 | 21 | 20 | 21 | 180 | 34 | $5.3 \times 10^5$ |
| | WCS417r | 127 | 19 | 30 | 29 | 205 | 38 | $6.2 \times 10^5$ |

[a]ISR was induced by transferring 2-week-old seedlings to soil containing *P. fluorescens* WCS417r bacteria at $5 \times 10^7$ cfu g$^{-1}$. Three-week-old plants were challenge-inoculated by applying 3 µl droplets to the leaves of a spore suspension of *P. parasitica* WACO9 at $5 \times 10^4$ spores ml$^{-1}$. At 11 days after challenge inoculation, disease symptoms were scored and spores were isolated and counted. Asterisks indicate statistically significant different distributions of the disease-severity classes compared to the control treatments (Chi-square, $P < 0.05$).

[b]Disease rating was expressed as intensity of sporulation on each leaf: 0, no sporulation; 1, <50% of the leaf area covered by sporangia; 2, >50% of the leaf area covered by sporangia; 3, heavily covered with sporangia, with additional chlorosis and leaf collapse.

[c]FW = fresh weight.

In this plant–pathogen interaction, induced resistance was expressed both as a reduction in symptom severity and a reduction of sporulation of the pathogen. In Col (*ISR1/ISR1*), treatment of the roots with WCS417r bacteria resulted in substantial induced protection against *P. parasitica* (Table 3). In contrast, ecotype Ws (*isr1/isr1*) did not develop resistance against this pathogen after treatment of the roots with WCS417r. Thus, the *ISR1* locus is not only involved in induced resistance against the pathogenic bacterium *Pst*, but also against pathogenic oomycete *P. parasitica*. However, non-induced Ws plants infected with *P. parasitica* did not exhibit enhanced disease susceptibility compared to Col (Table 3). Apparently, the *ISR1* locus does not contribute to basal resistance against *P. parasitica*, although it is involved in the regulation of ISR against this pathogen.

## Physiological characterisation of the *ISR1* phenotype

As described above, the *ISR1* locus in Arabidopsis is involved in ISR against different pathogens as well as specific basal resistance against *Pst*, whereas it does not contribute to basal resistance against the oomycetous pathogen *P. parasitica*. Interestingly, the ethylene response mutant *etr1-1* and the JA response mutant *jar1-1* exhibited a similar phenotype as ecotypes RLD and Ws, in that they were blocked in WCS417r-mediated ISR, affected in basal resistance against *Pst*, but unaffected in basal resistance against *P. parasitica* (Pieterse et al., 1998; Thomma et al., 1998; Ton et al., 1999). This resemblance in phenotypic characteristics suggests that the *ISR1* locus could be involved in the JA/ethylene-dependent signalling. Indeed, recent results indicate that RLD (*isr1/isr1*) and Ws (*isr1/isr1*) showed reduced sensitivity to ethylene in comparison to Col (*ISR1/ISR1*), as determined by both physiological responses and ethylene-dependent gene expression. This reduced sensitivity to ethylene cosegregated with the recessive alleles at the *ISR1* locus (Ton et al., 2000). Therefore, it is proposed that the *ISR1* locus encodes a component of the ethylene-response pathway that plays a role in ethylene-dependent resistance responses. Future research will be directed towards cloning the gene at the *ISR1* locus, and characterising it in relation to the role of ethylene in basal resistance against pathogens and rhizobacteria-mediated ISR.

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