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Research Note

A Disease Resistance Gene in *Arabidopsis* with Specificity for the *avrPph3* Gene of *Pseudomonas syringae* pv. *phaseolicola*

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The avirulence gene *avrPph3* from *Pseudomonas syringae* pv. *phaseolicola* was tested for its ability to convert virulent *P. syringae* pv. *tomato* strain DC3000 to avirulence on *Arabidopsis*. In F_2 plants from a cross between resistant and susceptible ecotypes, the ratio of resistant to susceptible plants was approximately 3:1, indicating that resistance to DC3000(*avrPph3*) is determined by a single dominant locus, which we have designated *RPS5*. *RPS5* was mapped to chromosome 1, between restriction fragment length polymorphism markers m241 and g3786.

Additional keywords: genetic mapping, nonhost resistance, recombinant inbred lines.

The gene-for-gene model was originally proposed to explain interactions between cultivars of a single plant species and races of a single pathogen species (Flor 1971). However, classical disease resistance (*R*) genes have recently been shown to be at least partially responsible for "nonhost" resistance. For example, Keen and Buzzel (1991) genetically identified four *R* gene loci in soybean that recognize four different avirulence (*avr*) genes isolated from the tomato pathogen *Pseudomonas syringae* pv. *tomato* (Kobayashi *et al.* 1989), which is not a pathogen of soybean. Whalen *et al.* (1988) demonstrated that pathovars of *Xanthomonas campestris* that are specific to bean, corn, or cotton could be converted to avirulence on their respective host plants by the addition of the *avrRxv* gene from *X. campestris* pv. *vesicatoria,* a pathogen of pepper. This indicates that each of these plant species possesses a resistance determinant with specificity for *avrRxv.* Recognition of *avr* genes by nonhost plant species appears to be common, as at least 10 different *P. syringae avr* genes are known to have this property (Kobayashi *et al.* 1989; Whalen *et al.* 1991; Dangl *et al.* 1992; Fillingham *et al.* 1992; Ronald *et al.* 1992; Innes *et al.* 1993a,b; Wood *et al.* 1994).

Such findings suggest that *R* genes and the interactions governing the specificity of resistance may be conserved among diverse plant species. Thus, it may be more expedient to isolate *R* genes and other components of the pathogen response signaling pathway in crop species by first identifying their homologs in *Arabidopsis* (Innes *et al.* 1993b). Here we report that *Arabidopsis* has a disease resistance locus that recognizes the *avrPph3* avirulence gene from the bean pathogen *P. syringae* pv. *phaseolicola* (Jenner *et al.* 1991).

We tested avirulence gene *avrPph3* (Jenner *et al.* 1991) for its ability to confer avirulence to *P. s.* pv. *tomato* strain DC3000 on ecotypes of *Arabidopsis.* A cosmid subclone bearing *avrPph3* designated pPPY424 by Fillingham *et al.* (1992) (a generous gift from J. Mansfield, Department of Biochemistry and Biological Sciences, Wye College, Ashford, Kent, U.K.) was digested to completion with *Bgl*II and then partially digested with *Hin*dIII to yield a 1.4-kb *Bgl*II-*Hin*dIII fragment containing *avrPph3.* The 1.4-kb partial-digest product was subcloned into the plasmid pVSP61, which contains an origin of replication from plasmid pVS1 of *P. aeruginosa* that functions in *P. syringae* (Itoh *et al.* 1984) and a second origin from pACYC184 for replication in *Escherichia coli.* Clones were transferred from *E. coli* to *P. s.* pv. *tomato* strain DC3000 by triparental mating as previously described (Ditta *et al.* 1980; Figurski and Helinski 1979). Gene *avrPph3* conferred on *Arabidopsis* a novel pattern of avirulence to DC3000. Specifically, ecotypes Columbia (Col-0) and Blanes (Bla-2) were resistant to DC3000(*avrPph3*). However, ecotype Landsberg-erecta (Ler) developed clear disease symptoms 4 days after inoculation, which were identical to those observed 4 days after a control inoculation with virulent DC3000. The avirulence differential between Col-0 and Ler is unique among those observed with any other *P. syringae avr* gene, suggesting that *avrPph3* is recognized by a resistance locus other than *RPM1/RPS3* (Bisgrove *et al.* 1994; Innes *et al.* 1993b) and *RPS2* (Kunkel *et al.* 1993). We have designated this resistance locus *RPS5,* for "resistance to *P. syringae* gene number 5" (*RPS4* of *Arabidopsis* confers resistance specific to an avirulence gene from *P. syringae* pv. *pisi;* M. Hinsch and B. Staskawicz, personal communication).

To confirm that disease symptoms on ecotype Ler resulted from increased pathogen growth relative to Bla-2 and Col-0, we monitored the growth of strain DC3000(*avrPph3*) in rosette leaves of the three ecotypes as described by Whalen *et al.* (1991) and Bisgrove *et al.* (1994). The growth of DC3000(*avrPph3*) and that of the virulent control strain DC3000(*avrB*::Ω) in the three ecotypes are shown in Figure 1. The control strain carries the *avrB* gene disrupted by an Ω

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fragment (Prentki and Krisch 1984) and hence its reversion to virulence (Innes *et al.* 1993b). In Ler, the growth of DC3000(*avrPph3*) and that of virulent DC3000 were indistinguishable, producing a final leaf population of approximately 1×10^8 cfu/cm^2 4 days after inoculation. However, in Bla-2 and Col-0, DC3000(*avrPph3*) growth was at least 100 times less than that of the virulent control strain 2 days after inoculation, and the bacterial populations did not exceed 1×10^5 cfu/cm^2 by the end of the monitoring period.

To examine the dominance of *RPS5*-mediated resistance, we monitored the growth of DC3000(*avrPph3*) in rosette leaves of F_1 plants from Col-0 × Ler and Bla-2 × Ler crosses. The results shown in Figure 1 suggest that *RPS5*-mediated resistance is completely dominant. Within 2 days after inoculation DC3000(*avrPph3*) growth in Bla-2 × Ler F_1 plants was almost 100 times lower than that of the virulent control, and in Col-0 × Ler F_1 plants it was clearly 100 times lower. By the end of the 5-day monitoring period DC3000(*avrPph3*) growth did not significantly exceed or remained below 1×10^5 cfu/cm^2, which is similar to results obtained with the resistant parents alone.

We used DC3000(*avrPph3*) to dip-inoculate F_1 progeny from an *rps3-1* mutant of Col-0 crossed with Ler and from Bla-2 crossed with Ler. In both crosses, all F_1 progeny were resistant, confirming that *RPS5*-mediated resistance is a dominant trait and independent of *RPS3*. To determine if *RPS5* is a single dominant gene, we studied F_2 populations derived from the same Col-0(*rps3-1*) × Ler and Bla-2 × Ler crosses to analyze for resistance to DC3000(*avrPph3*). The ratio of resistant to susceptible plants was approximately 3:1 in both F_2 populations, indicating that *RPS5* behaves as a single dominant gene (Table 1).

We used recombinant inbred (RI) lines generated from a cross between ecotypes Col-0 and Ler (Lister and Dean 1993) to map *RPS5*. Approximately 100 genetic markers have been accurately scored in 100 of these RI lines, and these 100 lines were dip-inoculated with DC3000(*avrPph3*) and scored as either the Col-0 (resistant) genotype or the Ler (susceptible) genotype. A 1:1 ratio of resistant to susceptible lines was observed, as expected (Table 1). We used Macintosh version 1.0 of the MAPMAKER linkage analysis program (Lander *et al.* 1987) to compare the *RPS5* score for each line to those of

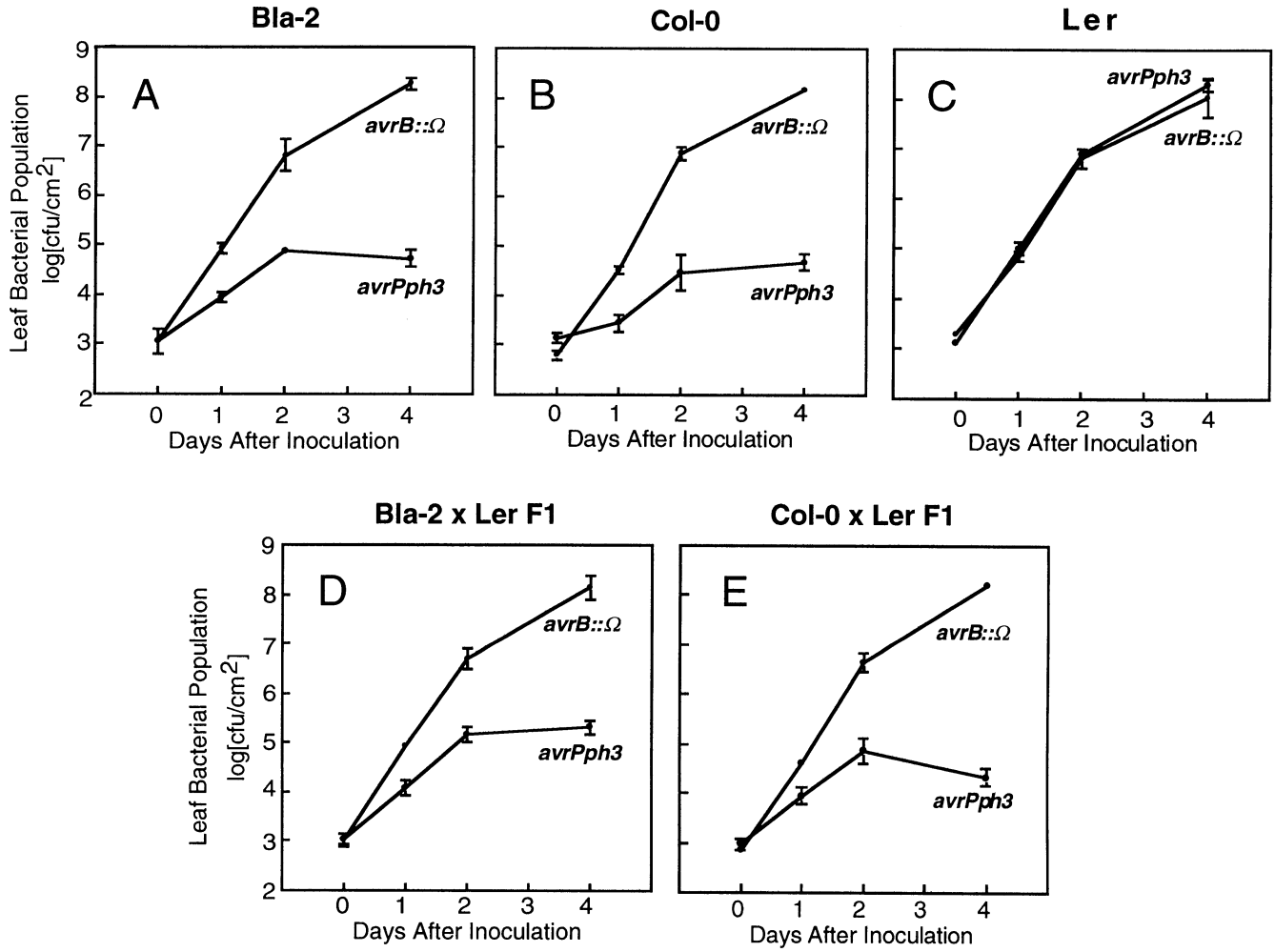

Fig. 1. Growth of *Pseudomonas syringae* pv. *tomato* strains within leaves of *Arabidopsis*. **A–C,** Bacterial growth in leaves of the resistant ecotypes Blanes (Bla-2) and Columbia (Col-0) and the susceptible ecotype Landsberg-erecta (Ler) inoculated by vacuum infiltration with strain DC3000 carrying *avrPph3* or an *avrB* gene disrupted with an Ω fragment that served as a virulent control strain. **D** and **E,** Bacterial growth in leaves of Bla-2 × Ler and Col-0 × Ler F_1 plants that were similarly inoculated. Col-0 and Ler used in **B–E** were the parents of the recombinant inbred lines. Growth of bacteria within the leaves was monitored over a 4-day time course. Each data point represents the mean ± standard error of three samples.

the markers scored in the RI lines. This enabled us to assign *RPS5* to chromosome 1, between markers m241 and g3786 (Fig. 2). This analysis also enabled us to predict that 12 of the RI lines should have recombination events between m241 and g3786. To verify that these 12 RI lines had recombination events in this interval, we hybridized ^{32}P-labeled m241 and g3786 DNA to Southern blots of DNA from the putative recombinants. We verified all 12 recombinant RI lines: four that were recombinant between m241 and *RPS5* and eight that were recombinant between *RPS5* and g3786. The approximate map distances and marker orientations derived from these recombination frequencies are shown in Figure 2. Because these data were obtained from RI lines, rather than an F_2 population, the genetic map distances were calculated by the formula of Haldane and Waddington (1931): $r = R/(2 - 2R)$, where r is the map distance, and R is the fraction of recombinants.

Gene *avrPph3* is recognized in a gene-for-gene manner by the dominant *R3* locus of the bean cultivar Tendergreen (Jenner *et al.* 1991). Fillingham *et al.* (1992) reported that *avrPph3* also conferred avirulence to *P. s.* pv. *pisi* races 1, 5, and 7 on all seven pea cultivars tested. Combined with our results, these observations suggest *Arabidopsis,* pea, and bean contain an *R* gene that is specific for *avrPph3.* Why functional specificity for *avrPph3* has been conserved between these diverse plant species is unclear. The *avrPph3* gene or a similar sequence has not been detected beyond *P. s.* pv. *phaseolicola* races 3, 4, and 8 (Jenner *et al.* 1991). Similarity to other *avr* genes outside the putative *hrp* box is not observed (Jenner *et al.* 1991). *RPS5* may confer resistance specific to other unknown avirulence genes. If *avrPph3* produced an elicitor similar to those produced by unknown avirulence genes specific to *RPS5,* then *avrPph3* specificity may have been conserved as well. An example of an *R* gene with such a dual specificity is the *RPM1/RPS3* resistance gene of *Arabidopsis* (Bisgrove *et al.* 1994).

Table 1. Segregation of *RPS5* in F_2 populations and in recombinant inbred lines of *Arabidopsis*

	Number of plants			
Cross[a]	Resistant	Susceptible	Total	χ^2
Bla-2 × Ler	442	131	573	1.4*[b]
Col-0, *rpm1-3* × Ler	325	125	450	1.9*[b]
Recombinant inbred lines	50	50	100	0.0*[c]

[a] Crosses between the resistant ecotypes Blanes (Bla-2) and Columbia (Col-0) and the susceptible ecotype Landsberg-erecta (Ler)
[b] χ^2 values calculated for a 3:1 segregation ratio of resistant to susceptible F_2 plants.
[c] χ^2 value calculated for a 1:1 ratio of resistant to susceptible plants.

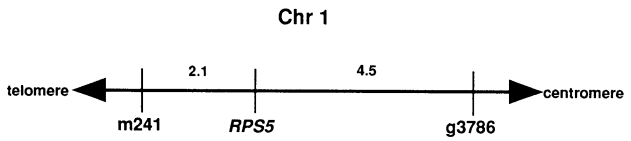

Fig. 2. Genetic map position of *RPS5.* The numbers above the horizontal bar indicate the approximate map distance in centimorgans between *RPS5* and restriction fragment length polymorphism markers m241 and g3786. The map distances from m241 to *RPS5* and *RPS5* to g3786 are based on scoring 97 and 93 recombinant inbred lines, respectively.

Determination of whether the mechanism of *avrPph3* recognition is the same in bean, pea, and *Arabidopsis* must await cloning of a corresponding *R* gene. Convergent evolution of these species to cope with persistent exposure to "*avrPph3*-like" elicitors may have resulted in dissimilar modes of recognition of *avrPph3,* in which case the *R* genes that mediate *avrPph3* recognition need not be true homologs. However, if the functional conservation is the result of *R* gene conservation during speciation, isolation of *RPS5* from *Arabidopsis* should facilitate isolation of its homologs in bean and pea.

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