The Plant Cell, Vol. 5, 865–875, August 1993 © 1993 American Society of Plant Physiologists

RPS2, an Arabidopsis Disease Resistance Locus Specifying Recognition of *Pseudomonas syringae* Strains Expressing the Avirulence Gene *avrRpt2*

Barbara N. Kunkel,[a,1] **Andrew F. Bent,**[a,1] **Douglas Dahlbeck,**[a,1] **Roger W. Innes,**[a,b,2]
and Brian J. Staskawicz[a,1,3]

[a] Department of Plant Pathology, University of California, Berkeley, California 94720
[b] Department of Biology, Indiana University, Bloomington, Indiana 47401

A molecular genetic approach was used to identify and characterize plant genes that control bacterial disease resistance in Arabidopsis. A screen for mutants with altered resistance to the bacterial pathogen *Pseudomonas syringae* pv. *tomato* (*Pst*) expressing the avirulence gene *avrRpt2* resulted in the isolation of four susceptible *rps* (resistance to *P. syringae*) mutants. The *rps* mutants lost resistance specifically to bacterial strains expressing *avrRpt2* as they retained resistance to *Pst* strains expressing the avirulence genes *avrB* or *avrRpm1*. Genetic analysis indicated that in each of the four *rps* mutants, susceptibility was due to a single mutation mapping to the same locus on chromosome 4. Identification of a resistance locus with specificity for a single bacterial avirulence gene suggests that this locus, designated *RPS2*, controls specific recognition of bacteria expressing the avirulence gene *avrRpt2*. Ecotype Wü-0, a naturally occurring line that is susceptible to *Pst* strains expressing *avrRpt2*, appears to lack a functional allele at *RPS2*, demonstrating that there is natural variation at the *RPS2* locus among wild populations of Arabidopsis.

INTRODUCTION

Plant resistance to disease caused by phytopathogenic organisms is often triggered by specific recognition of a given pathogen. This recognition leads to the rapid induction of plant defense mechanisms that limit multiplication and spread of the pathogen within the plant (Lamb et al., 1989). One common component of plant defense responses is the hypersensitive response (HR), which involves rapid, localized cell death and tissue necrosis at the site of infection (Klement, 1982). Classical genetic analyses of plant pathogens and their hosts have demonstrated that, in many cases, pathogen recognition is determined by single, dominant or semidominant resistance genes in the host with specificity for single, dominant avirulence (*avr*) genes in the pathogen (Flor, 1971; Keen, 1990). Pathogen recognition (and subsequent expression of resistance) occurs only when resistance and avirulence genes with matched specificity are present in the interacting organisms.

The mechanisms by which these "gene-for-gene" interactions govern pathogen recognition are not understood, nor is it clear how pathogen recognition triggers the expression of plant defense responses. Two avirulence genes are known to govern the synthesis of extracellular compounds that specifically elicit expression of defense responses in plants containing the corresponding resistance gene (Keen et al., 1990; Van den Ackerveken et al., 1992). The only plant resistance gene that has been cloned and characterized is *HM1*, a gene from maize that determines resistance against strains of the fungal pathogen *Cochliobolus carbonum* that produce HC-toxin (Johal and Briggs, 1992). The discovery that *HM1* encodes a reductase that inactivates HC-toxin (Johal and Briggs, 1992; Meeley et al., 1992) provides one example for how a single plant gene can determine race-specific resistance.

To facilitate the identification and characterization of plant genes controlling disease resistance, we and others have recently begun studying disease resistance in Arabidopsis. Arabidopsis has been established as a model host for several bacterial, viral, fungal, and nematode pathogens (reviewed in Dangl, 1992). We are focusing on the interactions between Arabidopsis and the bacterial pathogen *Pseudomonas syringae*, a causal agent of leaf spotting diseases (Schroth et al., 1981). Both virulent strains of *P. syringae* pv. *tomato* (*Pst*) that are able to cause disease on Arabidopsis and avirulent strains that elicit resistance in Arabidopsis have been identified (Davis et al., 1991; Debener et al., 1991; Dong et al., 1991; Whalen et al., 1991). Additionally, the bacterial avirulence genes *avrRpt2*, *avrB*, and *avrRpm1* have been shown to play a role in Arabidopsis–*P. syringae* interactions (Debener et al., 1991;

---

[1] Current address: Department of Plant Biology, University of California, Berkeley, CA 94720.
[2] Current address: Department of Biology, Indiana University, Bloomington, IN 47401.
[3] To whom correspondence should be addressed.

Dong et al., 1991; Whalen et al., 1991; Bent et al., 1992; Dangl et al., 1992). When introduced into *Pst*, any of these three avirulence genes converts a normally virulent strain, such as *Pst* strain DC3000, into an avirulent one that induces an HR and is no longer capable of causing disease on Arabidopsis ecotype Columbia (Col-0). Strains carrying the cloned avirulence genes remain virulent on other naturally occurring Arabidopsis ecotypes, demonstrating that the avirulent phenotype controlled by these pathogen genes is plant genotype specific (Debener et al., 1991; Whalen et al., 1991; Innes et al., 1993b).

Molecular analysis of *avrRpt2* has revealed that this avirulence gene encodes a single putative polypeptide (Innes et al., 1993a). In accordance with the gene-for-gene hypothesis of disease resistance (Flor, 1971; Keen, 1990), we set out to identify a corresponding locus in the resistant ecotype Col-0 that controls resistance to *Pst* strains expressing *avrRpt2*. The availability of isogenic *P. syringae* strains differing only in the presence or absence of the cloned *avrRpt2* gene (Whalen et al., 1991) has facilitated our study of the genetic basis of *avrRpt2*-mediated resistance. We utilized two different genetic approaches to identify plant genes that control disease resistance in Arabidopsis to *P. syringae* strains expressing *avrRpt2*: mutational analysis of resistance in ecotype Col-0 and genetic analysis of the natural variation that exists among Arabidopsis ecotypes. Here, we describe the identification and initial characterization of an Arabidopsis disease resistance locus, designated *RPS2*, that controls specific recognition of *P. syringae* strains expressing the avirulence gene *avrRpt2*. We also present evidence for natural variation at the *RPS2* locus among wild isolates of Arabidopsis.

RESULTS

Isolation of Arabidopsis Mutants with Altered Resistance to *P. syringae* Expressing *avrRpt2*

To facilitate the isolation of Arabidopsis mutants with altered resistance to *P. syringae* expressing *avrRpt2*, we utilized a procedure that allows the efficient inoculation of large numbers of plants. This inoculation procedure involved dipping entire leaf rosettes into a bacterial suspension containing the surfactant Silwet L-77 (Whalen et al., 1991). As is shown in Figure 1, Arabidopsis ecotype Columbia (Col-0) plants inoculated by this method with the virulent *Pst* strain DC3000 exhibited disease symptoms consisting of many small, individual necrotic lesions, each surrounded by a halo of chlorosis. In contrast, wild-type Col-0 plants inoculated by this method with *Pst* strain DC3000 expressing the avirulence gene *avrRpt2* (*Pst* DC3000[*avrRpt2*]) exhibited virtually no disease symptoms (Figure 1). Because infection by *Pst* is nonsystemic, diseased plants typically outgrew the infection and set seed, allowing recovery of progeny.

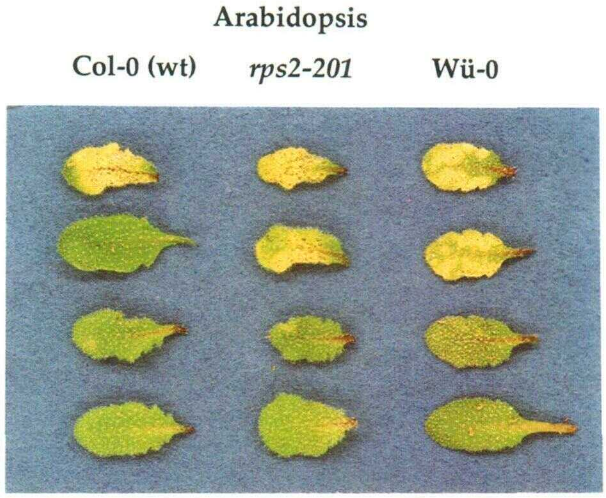

Figure 1. Phenotypes of Wild-Type Col-0, Susceptible Mutant *rps2*–201, and Ecotype Wü-0 Inoculated with *Pst* Strains Expressing Different Avirulence Genes.

Leaves of Arabidopsis plants are shown 4 days after inoculation with the following *Pst* DC3000 strains: top row, DC3000; second row, DC3000(*avrRpt2*); third row, DC3000(*avrB*); and fourth row, DC3000(*avrRpm1*). Plants were inoculated by dipping into bacterial suspensions containing the surfactant Silwet L-77 (see Methods).

Arabidopsis mutants with altered resistance to *P. syringae* were identified by using the above procedure to inoculate *Pst* DC3000(*avrRpt2*) onto populations of M_2 plants derived from seed of the resistant ecotype Col-0 that had been mutagenized with diepoxybutane. Of ~7500 M_2 plants tested, four plants were found to be susceptible to *Pst* DC3000(*avrRpt2*) upon retesting in the M_3 generation. These plants were confirmed to be true mutants (i.e., derived from ecotype Col-0 and not from a susceptible ecotype) by analysis of restriction fragment length polymorphism (RFLP) markers (data not shown). Interestingly, two of the mutants were initially isolated as heterozygotes, as revealed by the observation that self-progeny of these mutant lines segregated for resistance in the M_3 generation. This suggested that these mutations were partially dominant. The susceptible mutants were designated *rps* mutants (for resistance to P. syringae). One of the *rps* mutants, *rps2-201*, was chosen for more extensive characterization.

Characterization of Susceptible Mutant *rps2-201*

The *rps2-201* mutant line exhibited severe disease symptoms when inoculated with *Pst* DC3000(*avrRpt2*) (Figure 1). As summarized in Table 1, the susceptible phenotype was also apparent in mutant plants inoculated by pipette infiltration. Wild-type, resistant Col-0 plants inoculated with 10^6 colony-forming units (cfu)/mL *Pst* DC3000(*avrRpt2*) exhibited no disease symptoms (Table 1). However, within 5 days after inoculation with *Pst* DC3000(*avrRpt2*), *rps2-201* mutant leaves developed

Table 1. Phenotypes of Susceptible Mutant *rps2-201* and Ecotypes Wü-0 and No-0 Inoculated with Several *Pst* Strains[a]

	Arabidopsis				
Pst	Col-0 (wt)	*rps2-201*	Col-0 × *rps2-201* F_1	Wü-0	No-0
DC3000[b]	S 4.3 ± 0.07 *n* = 191	S 4.2 ± 0.10 *n* = 111	S 4.0 ± 0.36 *n* = 15	S 4.3 ± 0.13 *n* = 34	S 3.9 ± 0.20 *n* = 34
DC3000(*avrRpt2*)	R 1.8 ± 0.10 *n* = 126	S 4.3 ± 0.09 *n* = 114	S 3.8 ± 0.36 *n* = 17	S 3.2 ± 0.18[c] *n* = 37	R 2.0 ± 0.27 *n* = 26
DC3000(*avrB*)	R 2.0 ± 0.17 *n* = 64	R 1.9 ± 0.23 *n* = 29	R 1.2 ± 0.2 *n* = 5	R 1.2 ± 0.14 *n* = 16	nt
DC3000(*avrRpm1*)	R 1.3 ± 0.16 *n* = 15	R 1.6 ± 0.33 *n* = 14	nt	R 1.2 ± 0.09 *n* = 19	nt

[a] Disease symptoms were scored 5 days after pipette infiltration with bacteria at 10^6 cfu/mL. Disease symptoms of each plant were rated on a scale of 1 (no symptoms) to 5 (inoculated region entirely necrotic). Disease scores are mean ± 1 SEM; mean disease scores ≥ 3.0 = susceptible (S); mean disease scores ≤ 2.0 = resistant (R); *n* = sample size; nt = not tested.

[b] *Pst* strain DC3000 not expressing a cloned avirulence gene carried plasmid pLAFR3 or pLH12Ω (See Methods).

[c] Disease scores produced by *Pst* DC3000(*avrRpt2*) on Wü-0 differed significantly from those produced by *Pst* DC3000, as determined by the Wilcoxon signed rank test ($P < .01$).

extensive, gray-brown necrotic lesions surrounded by a halo of chlorosis. The disease symptoms were visually indistinguishable from those produced by *Pst* DC3000 on wild-type Col-0 or *rps2-201* mutant plants (Figure 1; Table 1).

To determine whether the susceptible phenotype of the mutant reflected the level of bacterial growth within the plant, growth of *Pst* DC3000(*avrRpt2*) was monitored in *rps2-201* mutants. As shown in Figure 2, *Pst* DC3000(*avrRpt2*) typically grew to high levels in mutant plants, obtaining a final concentration of 10^7 to 10^8 cfu/cm^2 of leaf tissue. This level of bacterial growth is characteristic of susceptible interactions (Whalen et al., 1991). This was in contrast to the limited growth of the same *Pst* DC3000(*avrRpt2*) strain in resistant, wild-type Col-0 plants, which obtained a final concentration of only 10^4 to 10^5 cfu/cm^2 (Figure 2).

The susceptible phenotype of the *rps2-201* mutant line was also evident in the inability of mutant plants to elaborate a visible HR when inoculated with high levels of *P. syringae* strains expressing *avrRpt2*. As summarized in Table 2 and Figure 3, wild-type Col-0 plants inoculated with high levels of *P. syringae* strains expressing *avrRpt2* exhibited tissue collapse and necrosis in the inoculated region of the leaf within 24 hr after infiltration. Inoculation with *P. syringae* strains lacking *avrRpt2* did not induce tissue collapse on Col-0 (Table 2), confirming that the ability to elicit an HR on Col-0 was conferred by the presence of the *avrRpt2* gene. In contrast to the response of wild-type Col-0, *rps2-201* mutant leaves inoculated with high levels of *P. syringae* strains expressing *avrRpt2* did not exhibit an HR (Table 2; Figure 3).

To determine whether *rps2-201* mutants had lost resistance to *Pst* strains expressing other avirulence genes, we tested

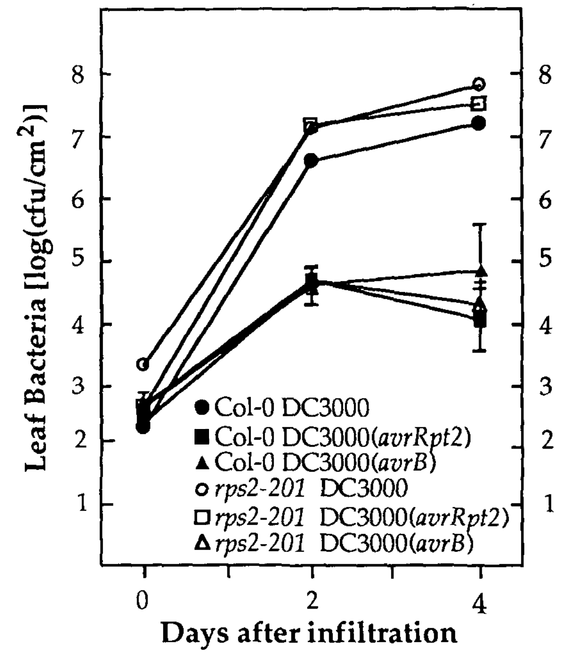

Figure 2. Growth of *Pst* DC3000 Strains in Susceptible Mutant *rps2-201*.

Concentrations of bacteria in plant leaves were assayed 0, 2, and 4 days after inoculation (see Methods). Data points represent the mean of three replicate experiments ± SEM. The avirulence gene clones were carried on vector pVSP61. The *Pst* DC3000 strain not expressing a cloned avirulence gene carried plasmid pVSP61 containing no insert. Bacterial growth was monitored as described in Methods. Similar results (not shown) were obtained in a second, independent experiment.

Table 2. HR of Susceptible Mutant *rps2-201* and Ecotypes Wü-0 and No-0[a]

	Arabidopsis				
P. syringae Strain	Col-0 (wt)	*rps2-201*	Col-0 × *rps2-201* F_1	Wü-0	No-0
P. syringae[b]	No HR H1.0 ± 0.01 *n* = 66	No HR H1.1 ± 0.04 *n* = 72	No HR H1.2 ± 0.11 *n* = 21	No HR H1.0 ±0.04 *n* = 52	No HR H1.0 ± 0.03 *n* = 18
P. syringae (*avrRpt2*)	HR H4.2 ± 0.08 *n* = 169	No HR H1.3 ± 0.05 *n* = 132	HR H2.4 ± 0.23 *n* = 26	No HR H1.0 ± 0.03 *n* = 69	HR H3.7 ± 0.22 *n* = 28
P. syringae (*avrB*)	HR H2.7 ± 0.11 *n* = 94	HR H2.7 ± 0.11 *n* = 63	HR H3.5 ± 0.34 *n* = 6	HR H1.9 ± 0.18 *n* = 29	nt
P. syringae (*avrRpm1*)	HR H2.8 ± 0.19 *n* = 26	HR H2.5 ± 0.28 *n* = 13	nt	HR H2.5 ± 0.22 *n* = 26	nt

[a] HR scored ~24 hr after pipette infiltration with either *Pst* DC3000 strains at 2 × 10^7 or *P. syringae* pv. *phaseolicola* strains at 10^8 cfu/mL (see Methods). HR ratings are on a scale of H1 (no tissue collapse) to H5 (full collapse of inoculated region). Ratings are presented as mean ± 1 SEM; HR scores ≥ H1.9 = HR; HR scores ≤ H1.5 = No HR; *n* = sample size; nt = not tested.

[b] *P. syringae* strains not expressing a cloned avirulence gene carried plasmid pLAFR3 or pLH12Ω (see Methods).

mutant plants for resistance to *Pst* DC3000 strains expressing *avrB* or *avrRpm1*. The results of L-77 inoculation (Figure 1), pipette infiltration (Table 1), and bacterial growth experiments (Figure 2) demonstrated that *rps2-201* mutant plants retained resistance to *Pst* strain DC3000 expressing *avrB* or *avrRpm1*, indicating that loss of resistance in the mutant was specific to *Pst* DC3000(*avrRpt2*). Mutant *rps2-201* plants also retained the ability to elaborate an HR when inoculated with high levels of *P. syringae* strains expressing *avrB* or *avrRpm1* (Table 2). These results suggest that the defect in the *rps2-201* mutant line is in its ability to specifically recognize bacteria expressing *avrRpt2*.

Genetic Analysis of Mutant *rps2-201*

To determine the genetic basis of susceptibility in the *rps2-201* mutant line, we performed reciprocal crosses of the mutant to the wild-type Col-0 parent line and to a second resistant ecotype, Nossen (No-0; Table 1). The F_1 progeny from these crosses were resistant to *Pst* DC3000(*avrRpt2*) when inoculated by the L-77 dipping procedure, as illustrated in Table 3. However, when assayed for resistance by pipette infiltration with 10^6 cfu/mL, the F_1 progeny were susceptible to *Pst* DC3000(*avrRpt2*) (Table 1; data not shown for *rps2-201* × No-0 F_1). When challenged with a lower dose of inoculum (i.e., 10^5 cfu/mL), F_1 plants exhibited no disease symptoms, whereas

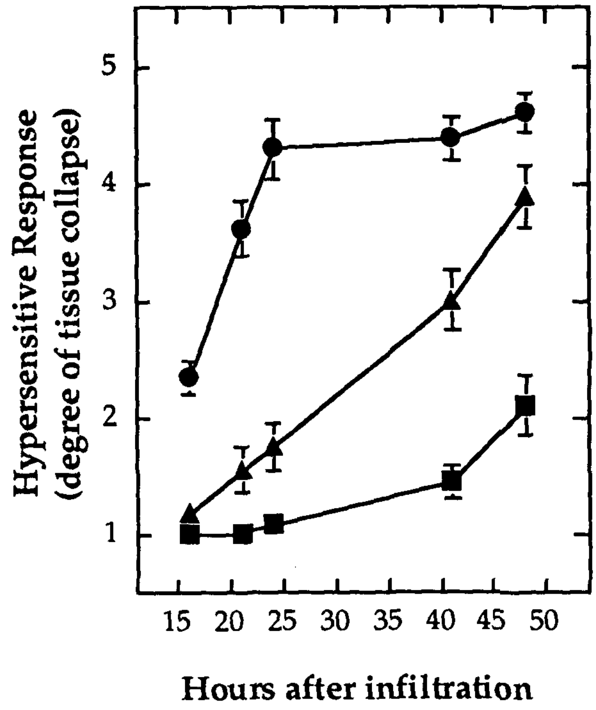

Figure 3. Development of the HR in *rps2-201* × Col-0 F_1 Heterozygotes.

The HR in Col-0 (wt) (●), *rps2-201* × Col-0 F_1 (▲), and *rps2-201* (■) plants was scored at the indicated times after pipette infiltration with 10^8 cfu/mL *P. syringae* pv. *phaseolicola* (*Psp*) strain 3121 carrying *avrRpt2* on plasmid pLH12 (see Methods). HR severity was judged visually on a scale of H1 (no tissue collapse) to H5 (full collapse of inoculated region). Ratings are presented as mean ± 1 SEM. Plants inoculated with *Psp* 3121 not expressing *avrRpt2* did not exhibit tissue collapse during the time course of the experiment.

rps2-201 mutant plants were still susceptible (data not shown). These results indicated that the F_1 progeny exhibited an intermediate resistance phenotype that was sensitive to the dose of inoculum used to assay for resistance. Consistent with this intermediate resistance phenotype was the observation that F_1 progeny exhibited only a weak or delayed HR. In contrast to wild-type Col-0, which exhibited tissue collapse within 24 hr after inoculation with high levels of *P. syringae* strains expressing *avrRpt2*, the F_1 progeny did not exhibit strong tissue collapse until 40 to 48 hr after inoculation (Figure 3). The intermediate resistance phenotype of the F_1 heterozygotes indicated that the *rps2-201* mutation was semidominant relative to the wild-type allele. As shown in Table 3, progeny derived from reciprocal crosses were indistinguishable in their resistance phenotype, indicating that the *rps2-201* mutation exhibited no maternal effect.

Analysis of F_2 progeny from crosses of mutant *rps2-201* to wild-type Col-0 or No-0 demonstrated that resistance to *Pst* DC3000(*avrRpt2*) in ecotype Col-0 was inherited as a monogenic trait. Resistance in F_2 progeny inoculated with *Pst* DC3000(*avrRpt2*) by the L-77 dipping procedure segregated in a ratio of 3 resistant:1 susceptible (Table 3). These results were verified by conducting progeny analysis of 73 F_2 individuals from the Col-0 *gl1* × *rps2-201* cross and 140 F_2 individuals from the *rps2-201* × No-0 cross. As expected, the F_3 families from both crosses fell into three phenotypic classes in a ratio of 1 uniformly resistant (predicted genotype R/R):2 segregating for resistance (R/S):1 uniformly susceptible (S/S) when inoculated by the L-77 dipping procedure (Table 3). Furthermore, the inability to exhibit an HR after inoculation with *P. syringae* expressing *avrRpt2* cosegregated with the susceptible phenotype, as determined using the L-77 inoculation assay (data not shown). These results indicated that in the *rps2-201* mutant line susceptibility to *Pst* DC3000(*avrRpt2*) and the inability to exhibit an HR in response to *P. syringae* strains expressing *avrRpt2* are conferred by a single mutation. We have designated the locus defined by this previously unidentified mutation *RPS2*.

Phenotypic and Genetic Analysis of the Additional *rps* Mutants

During the course of our mutant screen, we isolated three additional *rps* mutants that were phenotypically very similar to *rps2-201*. These mutants, designated *rps2-202*, *rps2-203*, and *rps2-301*, were susceptible to *Pst* DC3000(*avrRpt2*) when inoculated by the L-77 dipping and pipette infiltration procedures. The *rps* mutants were also unable to elaborate an HR when inoculated with high levels of *P. syringae* strains expressing *avrRpt2*. The mutants retained resistance to *Pst* DC3000 strains expressing *avrB* or *avrRpm1*, indicating that, as for mutant *rps2-201*, susceptibility in these mutants was specific for *Pst* DC3000(*avrRpt2*) (data not shown).

To determine the genetic basis of susceptibility in the *rps2-202*, *rps2-203*, and *rps2-301* mutant lines, we crossed each of the mutants back to the Col-0 parent line. Like *rps2-201*, the F_1 progeny from these crosses were intermediate in their

Table 3. Genetic Analysis of Susceptible Mutant *rps2-201*

		Number of Plants					
Cross	Generation	Resistant[a]	Susceptible[a]		Total	χ^2[b]	p
rps2-201/rps2-201	F_1	5	0		5		
× *RPS2/RPS2* (Col-0)	F_2	86	24		110	0.59	0.44
RPS2 gl1/RPS2 gl1	F_1	5	0		5		
× *rps2-201/rps2-201*	F_2	97	43		140	2.44	0.12
rps2-201/rps2-201	F_1	5	0		5		
× *RPS2/RPS2* (No-0)	F_2	227	65		294	1.17	0.28
		Deduced F_2 Genotypes					
		R/R	R/S	S/S	Total	χ^2	p
RPS2 gl1/RPS2 gl1 × *rps2-201/rps2-201*	F_3 families[c]	16	39	18	73	0.45[d]	0.80
rps2-201/rps2-201 × *RPS2/RPS2* (No-0)	F_3 families[c]	33	68	39	140	0.63[d]	0.73

[a] Plants were inoculated by dipping into bacterial suspensions containing Silwet L-77.

[b] χ^2 values were calculated for a segregation ratio of 3 resistant:1 susceptible plant.

[c] F_3 families were obtained by allowing individual F_2 plants to self-fertilize. For progeny testing, a minimum of 12 individuals per F_3 family were tested.

[d] χ^2 values calculated for a segregation ratio of 1 homozygous resistant (R/R):2 heterozygous (R/S):1 homozygous susceptible (S/S).

resistance phenotype and sometimes exhibited mild disease symptoms consisting of a few isolated, necrotic lesions, as summarized in Table 4. These results indicated that the additional *rps* mutations are also semidominant relative to the wild-type allele and are consistent with the fact that the *rps2-202* and *rps2-203* mutants were initially isolated as heterozygotes. Segregation of resistance to *Pst* DC3000(*avrRpt2*) in the F_2 progeny from these crosses indicated that in at least two of the three additional *rps* mutant lines susceptibility was conferred by single mutations (Table 4).

To determine whether the genetic lesions in the *rps2-202*, *rps2-203*, and *rps2-301* mutants mapped to *RPS2*, we crossed homozygous lines of each of these mutants to *rps2-201* and assayed the resulting F_1 and F_2 progeny for resistance to *Pst* strain DC3000(*avrRpt2)* by the L-77 inoculation procedure. The lack of complementation in the F_1 progeny from these crosses suggested that *rps2-202*, *rps2-203*, and *rps2-301* were allelic to *rps2-201* (Table 4). However, as the semidominant nature of the *rps2* mutations complicates interpretation of the results of these complementation tests, we also scored the resistance phenotypes of F_2 progeny from these crosses to map the additional *rps* mutations with respect to *rps2-201.* The absence of resistant plants among the F_2 progeny from crosses between *rps2-201* and the *rps2-202* and *rps2-301* mutant lines indicated that *rps2-202* and *rps2-301* map to *RPS2* or to a very closely linked locus (Table 4). Likewise, the observation of only two resistant plants from a total of 399 F_2 progeny scored from the cross between *rps2-201* and *rps2-203* (Table 4) indicated that the *rps2-203* mutation also maps to *RPS2* or to a closely linked locus. The two resistant plants observed are most likely artifacts of the inoculation procedure; in control experiments, homozygous susceptible plants inoculated with *Pst*

Table 4. Genetic Analysis of *rps2* Mutants and Ecotype Wü-0

		Number of Plants				
Cross	Generation	Resistant[a]	Susceptible[a]	Total	χ^2[b]	p
RPS2 gl1/RPS2 gl1[c]	F_1	0	5[d]	5		
× *rps2-202/rps2-202*	F_2	151	62[d]	213	1.92	0.17
rps2-201 gl1/rps2-201 gl1	F_1	0	4	4		
× *rps2-202/rps2-202*	F_2	0	261	261		
RPS2 gl1/RPS2 gl1	F_1	6	0	6		
× *rps2-203/rps2-203*	F_2	102	58[d]	160	10.8[e]	< 0.05
rps2-201 gl1/rps2-201 gl1	F_1	0	2	2		
× *rps2-203/rps2-203*	F_2	2[f]	397	399		
RPS2/RPS2	F_1	2	0	0		
× *rps2-301/rps2-301*	F_2	48	23	71	2.04	0.15
rps2-201/rps2-201	F_1	0	3	3		
× *rps2-301/rps2-301*	F_2	0	29	29		
rps2-201 gl1/rps2-201 gl1	F_2	2[f]	342	344		
× *rps2-101/rps2-101*						
RPS2/RPS2	F_1	6	0	6		
× *rps2-204/rps2-204* (Wü-0)	F_2	91	34	125	0.32	0.57
rps2-201/rps2-201	F_1	0	5	5		
× *rps2-204/rps2-204* (Wü-0)	F_2	5[f]	269	274		

[a] Plants were inoculated by dipping into bacterial suspensions containing L-77. Plants were scored 4 to 5 days after inoculation and grouped into susceptible and resistant phenotypic classes based on whether or not they exhibited necrotic lesions.

[b] χ^2 values were calculated for a segregation ratio of 3 resistant:1 susceptible plant.

[c] In crosses in which one of the parents carried the glabrous (*gl1*) morphological marker, the F_1 progeny were wild type for trichome development and the F_2 progeny segregated in a ratio of 3 wild type:1 glabrous.

[d] *rps2-202/RPS2* and *rps2-203/RPS2* heterozygotes were intermediate in resistance, often exhibited mild disease symptoms consisting of a few isolated necrotic lesions, and were therefore often scored as susceptible.

[e] Segregation of resistance among the F_2 progeny from this cross deviated significantly from a ratio of 3 resistant:1 susceptible plant. However, given the semidominant nature of the *rps2-203* mutation, this is not inconsistent with susceptibility in the mutant line being conferred by a single mutation.

[f] F_3 progeny from these F_2 individuals were not available for analysis. However, in 12 of 12 other cases in which putatively resistant plants from predominantly susceptible F_2 populations were subjected to progeny analysis, the resistant F_2 plants gave rise to F_3 families that segregated 100% for susceptible plants or were determined to be seed contaminants (see Methods).

DC3000(*avrRpt2*) by the L-77 dipping procedure occasionally exhibited very mild or no disease symptoms (data not shown; see also Table 4, footnote f).

We obtained from G. Yu a fifth Col-0 *rps* mutant, *rps2-101*, whose phenotype closely resembled that of *rps2-201* (Yu et al., 1993). Analysis of F_2 progeny from a cross of *rps2-201* to *rps2-101* indicated that the *rps2-101* mutation also maps to *RPS2* or to a closely linked locus (Table 4).

Identification and Analysis of Susceptible Ecotype Wü-0

In addition to the mutational approach described above, we took advantage of the natural variation that exists among wild isolates of Arabidopsis to study the genetic basis of resistance to *P. syringae* expressing *avrRpt2*. We identified an ecotype of Arabidopsis, Würzburg (Wü-0), that is susceptible to *Pst* DC3000(*avrRpt2*) (Figure 1). Wü-0 plants inoculated with *Pst* DC3000(*avrRpt2*) by pipette infiltration with 10^6 cfu/mL *Pst* DC3000(*avrRpt2*) developed extensive, gray-brown necrotic lesions within 5 days after inoculation (Table 1). However, the disease symptoms produced by *Pst* DC3000(*avrRpt2*) on ecotype Wü-0 were not as severe as those produced by *Pst* DC3000 lacking *avrRpt2* (Table 1). This apparent differential susceptibility was also reflected in experiments that monitored the level of bacterial growth within the plant. *Pst* DC3000(*avrRpt2*) grew to levels of 7.1 ± 0.37 (mean[log(cfu/cm^2)] ± standard error) in Wü-0, obtaining a final population density 10-fold lower than that observed for *Pst* DC3000, which grew to levels of 8.0 ± 0.03 in Wü-0. These results suggested that Wü-0 may possess some residual *RPS2* resistance activity. However, this activity does not appear to be sufficient to result in phenotypic resistance. The susceptible phenotype of this ecotype was also associated with the inability of Wü-0 plants to elaborate a visible HR when inoculated with high levels of *P. syringae* strains expressing *avrRpt2* (Table 2). As was the case for the *rps2* mutants, susceptibility in ecotype Wü-0 was specific to strains expressing *avrRpt2*, since Wü-0 retained resistance to *Pst* DC3000 expressing *avrB* or *avrRpm1* (Figure 1; Table 1).

To examine the genetic basis of susceptibility in Wü-0, this line was crossed to ecotype Col-0. When inoculated by the L-77 dipping procedure, the resulting F_1 progeny were resistant to *Pst* DC3000(*avrRpt2*) (Table 4). Resistance in the F_2 progeny from this cross segregated as a monogenic trait (Table 4). To determine whether susceptibility to *Pst* DC3000(*avrRpt2*) in Wü-0 was due to an alteration at the *RPS2* locus, we crossed Wü-0 to the susceptible *rps2-201* mutant line. Among the F_2 progeny from this cross, we observed 269 susceptible individuals from a total of 274 F_2 plants scored (Table 4), suggesting that susceptibility in ecotype Wü-0 was due to a defect or alteration at *RPS2* or at a second, very closely linked locus. We have designated the susceptible *rps2* allele present in ecotype Wü-0 *rps2-204*.

The *RPS2* Locus Maps to Chromosome 4

Two different strategies, RFLP linkage analysis and RAPD analysis (Williams et al., 1990), were used to genetically map the *RPS2* locus. Utilizing progeny from an *rps2-201* × No-0 cross, 115 F_3 families that had been scored for their resistance phenotype (and were thus of known genotype; see Table 3) were used to map *RPS2* relative to selected RFLP markers positioned at intervals of 20 to 40 centiMorgans (cM) on each of the five chromosomes (Chang et al., 1988; Nam et al., 1989). Using this approach, we mapped *RPS2* to chromosome 4, in an ∼10-cM interval between the RFLP markers M557 and M600, as illustrated in Figure 4. We identified 1 recombinant between *RPS2* and M600 from 218 chromosomes scored and 21 recombinants between *RPS2* and M557 from 232 chromosomes scored. This places *RPS2* at distances of ∼0.5 cM and 10 cM from M600 and M557, respectively (Figure 4), as calculated using the Kosambi mapping function (Kosambi, 1944; see Methods).

Because none of the published or readily available RFLP markers reported to map in the *RPS2* region of chromosome 4 mapped to the interval between M557 and *RPS2* (data not shown), we employed RAPD analysis to identify additional closely linked molecular markers (Williams et al., 1990). One hundred ten primers were screened for their ability to differentiate mutant *rps2-201* and No-0 on the basis of the appearance

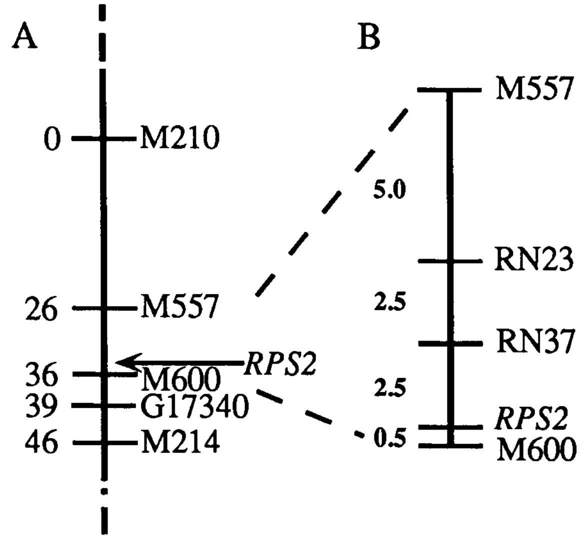

Figure 4. Map Position of *RPS2* on Chromosome 4 of Arabidopsis.

M210, M557, M600, G17340, and M214 are RFLP markers (Chang et al., 1988; Nam et al., 1989). RN23 and RN37 are RAPD markers (see text).

(A) The map distances (in cM) relative to marker M210 are indicated on the left.

(B) The genetic distances (in cM) separating the markers mapping between M557 and M600 are indicated and were calculated using multipoint analysis as described in Methods.

of unique polymerase chain reaction (PCR) products. Forty-five primers yielded PCR products that were present in ecotype No-0 but not in *rps2-201*. Two of these RAPD markers, designated RN23 and RN37, were shown to be closely linked to *RPS2* based on their segregation pattern in the F_2 progeny of the *rps2-201* × No-0 crosses. We identified three recombinants between RN37 and *RPS2* from 70 chromosomes scored, placing *RPS2* ~2.5 cM from RN37 (Figure 4). The recombinants between *RPS2* and RN37 and *RPS2* and M600 were mutually exclusive, indicating that *RPS2* maps between RN37 and M600.

DISCUSSION

Using a combination of genetic approaches, we have identified a disease resistance locus in Arabidopsis, designated *RPS2*, involved in pathogen recognition. Mutational analysis of disease resistance in ecotype Col-0 led to the identification of four *rps* mutants that lost resistance to *P. syringae* strains expressing the avirulence gene *avrRpt2*. The *rps* mutant lines were shown to be both susceptible to *Pst* strain DC3000 expressing *avrRpt2* and unable to mount a visible HR in response to *P. syringae* strains expressing *avrRpt2*. Loss of resistance in the mutants was specific for strains expressing *avrRpt2*, because they retained resistance to strains expressing other avirulence genes. Thus, the *rps* mutants were altered in their ability to specifically recognize *P. syringae* strains expressing *avrRpt2* and not in their overall ability to mount a successful defense response.

Detailed genetic analysis of one of the *rps* mutants, *rps2-201*, revealed that in this line both susceptibility to *Pst* DC3000 (*avrRpt2*) and the inability to exhibit a visible HR in response to *P. syringae* strains expressing *avrRpt2* were conferred by mutation at a single locus mapping to chromosome 4. Formal genetic demonstration of a single plant locus determining specific resistance to *Pst* strains expressing the cloned *avrRpt2* avirulence gene indicated that resistance in this Arabidopsis/*P. syringae* system is governed by a "gene-for-gene" interaction. Thus, the locus defined by *rps2-201*, which we have designated *RPS2*, behaves as a classical resistance locus (Flor, 1971) with specificity for *P. syringae* strains expressing the avirulence gene *avrRpt2*.

F_1 progeny from crosses between *rps2-201* and resistant wild-type Col-0 and No-0 lines exhibited an intermediate resistance phenotype, indicating that the *rps2-201* mutation was semidominant. F_1 plants inoculated by the L-77 dipping procedure were phenotypically resistant (Table 3), whereas the resistance phenotype of F_1 plants inoculated by pipette infiltration was dependent on the dose of inoculum (Table 1; data not shown). These results suggest that resistance in *rps2-201* heterozygotes can be overcome when challenged with large numbers of bacteria (i.e., in plants inoculated by pipette infiltration at 10^6 cfu/mL). Additionally, the *rps2-201* heterozygotes exhibited a delayed HR when inoculated with *P. syringae* strains expressing *avrRpt2*. Several other plant disease resistance loci have been reported to be semidominant (Torp and Jorgensen, 1986; Whalen et al., 1988; Dangl, 1992; Carland and Staskawicz, 1993). The semidominant nature of these resistance loci demonstrates that expression of resistance is sensitive to the number of functional copies of the resistance gene present. This observation may have implications pertaining to the structure and function of these plant resistance gene products.

To determine whether the additional *rps* mutants isolated in our screen mapped to *RPS2*, we crossed each of the susceptible mutants with *rps2-201* and scored the resulting F_1 and F_2 progeny for resistance to *Pst* DC3000(*avrRpt2*). Had any of these *rps* mutants mapped to a second, unlinked locus, we would have expected to recover ~9 of 16 resistant F_2 progeny. The absence of resistant plants among the F_2 progeny from crosses between *rps2-201* and the *rps2-202* and *rps2-301* mutant lines and the observation of only two resistant plants among the 399 F_2 progeny scored from a cross between *rps2-201* and *rps2-203* indicated that the *rps2-202*, *rps2-203*, and *rps2-301* mutations map to *RPS2* or to a closely linked locus (Table 4). Thus, in a screen for mutants with altered resistance to *Pst* strain DC3000 expressing *avrRpt2*, we apparently isolated four susceptible alleles of the *RPS2* disease resistance locus. At least three of these *rps2* alleles are independent, as they were isolated from separate lots of mutagenized seed (*rps2-202* and *rps2-203* were isolated from the same lot of mutagenized seed; see Methods).

Disease resistance loci have typically been identified and characterized utilizing crosses between naturally occurring susceptible and resistant plant lines. We have identified several naturally occurring lines of Arabidopsis, including ecotypes Po-1 (Whalen et al., 1991), Tsu-0, Zü-0 (R. Innes, unpublished results), and Wü-0 (Figure 1; Table 2), which are susceptible to *Pst* DC3000(*avrRpt2*). However, disease symptoms produced on Wü-0 following pipette inoculation of *Pst* DC3000(*avrRpt2*) appeared to be less severe than those produced by *Pst* DC3000 (Table 1). The most simple interpretation of this result is that Wü-0 carries a partially functional allele of *RPS2*. Wü-0, like the *rps2* mutants, retained resistance to *Pst* strains expressing other avirulence genes. Additionally, genetic analysis indicated that susceptibility in Wü-0 was due to an alteration at a single locus mapping to, or very close to, *RPS2*. The identification of a naturally occurring ecotype apparently lacking a fully functional allele of *RPS2* suggests that there is natural variation at the *RPS2* locus among wild isolates of Arabidopsis. Natural variation among Arabidopsis ecotypes has been observed for resistance to other pathogens, including *P. syringae* pv. *maculicola*, *Xanthomonas campestris*, *Peronospora parasitica*, and several plant viruses (Koch, 1990; Simpson and Johnson, 1990; Debener et al., 1991; Tsuji et al., 1991; Dangl, 1992; Simon et al., 1992).

The *RPS2* resistance locus described in this work may be the same as that defined by the *rps2-101* mutant identified by Yu et al. (1993). In genetic mapping experiments, *rps2-101* was placed at approximately the same chromosomal location as *rps2-201*, ~2 cM away from RFLP marker G17340 (Yu et al., 1993). To determine whether the *rps2-101* and *rps2-201* mutations mapped to the same locus, allelism tests were performed in both laboratories. Data obtained in both laboratories indicated that the two *rps* mutations map to the same or to closely linked loci (Table 4; Yu et al., 1993). However, definitive proof that any two *rps* mutations are allelic must await the cloning and sequencing of the wild-type and mutant *RPS2* loci.

Precedence for isolation of susceptible mutations mapping predominantly to a single disease resistance locus stems from the mutational analysis of resistance to powdery mildew in barley. Torp and Jorgensen (1986; Jorgensen, 1988) reported the isolation of 25 mutants with altered resistance to powdery mildew, 23 of which mapped to the *Ml-a12* resistance locus. More extensive analysis of resistance to *Pst* DC3000(*avrRpt2*) in Arabidopsis should allow us to determine if additional loci required for resistance can be identified by mutation.

The identification and initial characterization of an Arabidopsis resistance locus required for pathogen recognition provide a starting point for study of the molecular and biochemical mechanisms that control disease resistance in plants. The anticipated molecular cloning of the *RPS2* locus should contribute to our understanding of resistance gene function and will allow us to further address how specific pathogen recognition is achieved and how this recognition event ultimately results in the expression of disease resistance.

METHODS

Bacterial Strains and Plasmids

Pseudomonas syringae pv. *tomato* (*Pst*) strain DC3000 was obtained from D. Cuppels (Cuppels, 1986) and *P. syringae* pv. *phaseolicola* (*Psp*) strain 3121 was obtained from N. Panopoulos (Lindgren et al., 1986). *Pst* strain DC3000 and *Psp* strain 3121 expressing *avrRpt2* were constructed by the introduction of plasmids pABL18, pLH12 (Whalen et al., 1991), or pV288 by triparental mating using the helper plasmid pRK2013 (Figurski and Helinski, 1979). Plasmid pV288 carries the *avrRpt2* gene from *Pst* strain 1065 and was constructed by ligation of a 1.5-kb SalI fragment from pABL30 (Whalen et al., 1991) into the SalI site of pVSP61. pVSP61 is a 13.5-kb kanamycin-resistant plasmid vector that is highly stable in *Pseudomonas* strains due to the presence of the origin of replication from pVS1, a native plasmid of *P. aeruginosa* (W. Tucker, DNA Plant Technology Inc., Oakland, CA). Strains expressing *avrB* were constructed by the introduction of plasmid pPSG0002 (Staskawicz et al., 1987) or plasmid pVB01 by triparental mating. Plasmid pVB01 carries the *avrB* gene from *P. syringae* pv. *glycinea* race 0 (Tamaki et al., 1988) and was constructed by ligation of a 1.3-kb BglII/BamHI fragment from pPg0-13 (D. Dahlbeck, unpublished data) into the BamHI site of pVSP61. Strains expressing *avrRpm1* were constructed by the introduction of plasmid K48 by triparental mating (Debener et al., 1991). *P. syringae* strains not expressing cloned avirulence genes carried control plasmids pLAFR3 (vector without insert), pLH12Ω (an insertionally inactivated derivative of pLH12; Whalen et al., 1991), or pVSP61.

Plant Material, Growth Conditions, and Inoculation Procedures

Arabidopsis ecotypes Colombia (Col-0), Nossen (No-0), and Würzburg (Wü-0) were obtained from the Arabidopsis Information Service Seed Bank. Col-0 *gl1* was obtained from M. Estelle (Indiana University, Bloomington). Susceptible mutant *rps2-101* was provided by G. Yu (Massachusetts General Hospital, Boston). Arabidopsis plants were grown from seed in growth chambers under an 8-hr photoperiod at 24°C, as described previously (Whalen et al., 1991).

For mass inoculation of plants by dipping into bacterial suspensions containing surfactant, Arabidopsis seeds were sown in 3 1/2-inch-square pots at a density of 16 to 20 seedlings per pot and covered with fiberglass window screen held in place by a rubber band. Plants were grown under an 8-hr photoperiod at 24°C and 70 to 80% relative humidity under a mixture of fluorescent and incandescent lights at an intensity of 120 to 180 $\mu E\ m^{-2}\ sec^{-1}$. Entire leaf rosettes of 5-week-old plants were dipped into bacterial suspensions of 2 to 3 $\times\ 10^8$ colony-forming units (cfu)/mL in 10 mM $MgCl_2$ containing 0.02% Silwet L-77 (Union Carbide) and placed under plastic domes for 24 hr. Symptoms were scored 4 to 5 days after inoculation.

Pipette infiltrations to assay for disease resistance were performed as described previously (Whalen et al., 1991), using freshly grown bacteria resuspended in 10 mM $MgCl_2$ to an OD_{600} of 0.001 ($\sim 10^6$ cfu/mL). Plants were scored 5 days after inoculation. To assay for the hypersensitive response (HR), pipette infiltrations were conducted with bacteria resuspended in 10 mM $MgCl_2$ to an OD_{600} of 0.02 ($\sim 2 \times 10^7$ cfu/mL) for *Pst* DC3000 strains and to an OD_{600} of 0.1 ($\sim 10^8$ cfu/mL) for *Psp* 3121 strains. Leaves were scored for tissue collapse ~24 hr after inoculation and again at 40 to 48 hr after inoculation. Leaves infiltrated with *Psp* strain 3121 not expressing cloned avirulence genes did not exhibit tissue collapse during the time course of the experiment, thus allowing for the observation of a delayed HR. Bacterial growth within the plant was monitored as described previously (Whalen et al., 1991).

Induction and Isolation of Mutants

Stocks of diepoxybutane-mutagenized seeds of ecotype Col-0 were obtained from J. R. Ecker (University of Pennsylvania, Philadelphia) and M. Estelle (Indiana University). Arabidopsis seeds that had been hydrated overnight in water were soaked in 22 mM diepoxybutane (Sigma) for 4 hr at room temperature with continuous rocking. Lots of ~3000 M_1 seeds were planted separately to obtain independent populations of mutagenized M_2 generation seeds. Approximately 1000 seeds were screened from each of eight lots using the surfactant inoculation procedure described above. The mutants were isolated from three independent lots of mutagenized seed, with the exception of mutants *rps2-202* and *rps2-203*, which were isolated from the same seed lot and consequently may carry the same mutant allele. After identification, putative mutants were allowed to self-pollinate, and the resulting M_3 progeny were tested for the susceptible phenotype. Mutants *rps2-202* and *rps2-203* were initially isolated as heterozygotes; the self-progeny from these lines segregated for resistance in the M_3 generation. True-breeding, susceptible *rps2-202* and *rps2-203* lines used for further analysis were obtained from susceptible M_3 individuals following self-fertilization.

Genetic Analysis

Mutant *rps2-201* was back-crossed to wild-type Col-0, using both the parental wild-type Col-0 and Col-0 *gl1*, a marked line that lacks leaf and stem trichomes (glabrous; Koornneef et al., 1982). In crosses where the female parent carried the *gl1* mutation, all F_1 progeny had normal trichomes, and the F_2 progeny segregated in a ratio of 3 wild-type:1 glabrous plant. F_1 progeny from crosses between *rps2-201* and ecotypes No-0 and Wü-0 were verified as being true cross progeny by DNA gel blot analysis, using restriction fragment length polymorphism (RFLP) markers that reveal polymorphisms between the different ecotypes as probes. Allelism tests were performed by crossing the *rps* mutants to a marked *rps2-201* line carrying the *gl1* mutation, except for mutant *rps2-301*, which was crossed to an unmarked *rps2-201* line. Individual F_2 progeny were scored for their resistance phenotype by surfactant inoculation with *Pst* DC3000(*avrRpt2*). F_3 families were obtained by allowing individual F_2 plants to self-fertilize. For progeny testing, a minimum of 12 individuals per F_3 family were tested for their resistance phenotype using the surfactant inoculation procedure. F_2 plants that gave rise to F_3 families consisting of only resistant individuals were scored as homozygous resistant (*RPS2/RPS2*), F_2 plants that gave rise to F_3 families consisting of both resistant and susceptible individuals were scored as heterozygous (*RPS2/rps2*), and F_2 plants that gave rise to F_3 families consisting of only susceptible individuals were scored as homozygous susceptible (*rps2/rps2*). Among the putative resistant F_2 progeny derived from the cross of *rps2-201* × *rps2-101* were two additional resistant individuals not reported in Table 4. These individuals were judged to be contaminants based on their morphology, the fact that the two plants were located adjacent to each other in the same pot, and the finding that these two plants were homozygous resistant at the *RPS2* locus.

RFLP and RAPD linkage analyses were performed utilizing progeny from the cross between mutant *rps2-201* (Col-0 background) and the resistant ecotype No-0. The DNAs tested were isolated from 115 F_3 families (a minimum of 12 individuals per family) that had been scored for resistance to *Pst* DC3000(*avrRpt2*). RFLP markers were obtained from the laboratories of E. Meyerowitz and H. Goodman (Chang et al., 1988; Nam et al., 1989). Multipoint linkage analysis was performed using a Macintosh version of MapMaker (version V; Lander et al., 1987). Recombination frequencies from multipoint analysis were converted into map distances (centiMorgans) using the Kosambi function (Kosambi, 1944). Plant DNA was isolated according to the method of Tai and Tanksley (1990) with the following modifications: fresh plant tissue was frozen in liquid nitrogen and ground using either a mortar and pestle or an electric coffee grinder. DNA was isolated either from leaf tissue or from roots of seedlings that had been grown in liquid Gamborgs B-5 medium (Gamborg et al., 1968). Standard procedures for probe preparation and DNA gel blot hybridizations were followed (Maniatis et al., 1989).

One hundred eleven RAPD primers were obtained from B. Baker (U.S.Department of Agriculture Plant Gene Expression Center, Albany, CA). The two primers reported in this work are (5′ to 3′): p23, GGGCGGTTAA and p37, GGTACCAGAG. Polymerase chain reaction (PCR) reactions contained 50 mM KCl, 10 mM Tris-HCl, pH 8.3, 2 mM $MgCl_2$, 0.001% gelatin, 3% DMSO, 100 μM each of 4 dNTPs, 200 μM primer, 10 ng genomic DNA, and 1 unit of Taq Polymerase (Perkin-Elmer Cetus) in a 20-μL volume overlaid with 40 μL mineral oil. Amplification was performed in a thermal cycler (model PHC2; Techne, Inc., Princeton, NJ) or a thermal contoller (MJ Research, Watertown, MA) programmed for 10 cycles of 1 min at 94°C, 1 min at 35°C, 15 sec at 45°C, and 1 min, 45 sec at 72°C, followed by 35 cycles of 1 min at 92°C, 1 min at 35°C, 15 sec at 45°C, and 1 min, 45 sec at 72°C. Amplification products were resolved by electrophoresis on a gel of 1.5% Ultrapure agarose (GIBCO BRL) plus 1.5% NuSieve GTG agarose (FMC BioProducts, Rockland, ME). Primer p23 revealed a unique band in No-0 of 1.1 kb and primer p37 revealed a unique band of 0.7 kb in No-0. The bands of interest were cloned for further analysis by eluting the band from a gel, treating the eluted DNA with T4 DNA polymerase and dNTPs to fill in any recessed ends, and ligating the DNA into pBluescript KS+ (Stratagene) that had been linearized with restriction endonuclease EcoRV. To reproducibly score the p37 PCR reactions, the amplification products were resolved by electrophoresis, blotted onto Hybond N^+ membrane (Amersham), and probed with the cloned 0.7-kb p37 PCR product. The RAPD markers linked to *RPS2* derived from p23 and p37 were designated RN23 and RN37, respectively.

ACKNOWLEDGMENTS

We thank Sherryl Bisgrove for assistance with genetic analysis of *rps2-301*; Margaret Lin for assistance with phenotypic characterization of F_1 heterozygotes; Karen Century for construction of plasmid pVB01; Joseph Ecker, Mark Estelle, and William Tucker for biological materials; Frederick Ausubel and Guo-Liang Yu for communication of results prior to publication; and Kit Brown, John Salmeron, Kathy Swords, and Maureen Whalen for critical review of the manuscript. B. N. K. was a Department of Energy–Energy Biosciences Postdoctoral Research Fellow of the Life Sciences Research Foundation. A.F.B. and R.W.I. were National Science Foundation Postdoctoral Research Fellows in Plant Biology. This work was supported by Department of Energy Grant No. DE-FG03-88ER13917 to B.J.S. Work in the laboratory of R.W.I. was funded by National Institutes of Health Grant No. PHS R29 GM 46451.

Received January 28, 1993; accepted June 29, 1993.

REFERENCES

Bent, A.F., Innes, R.W., Ecker, J.R., and Staskawicz, B.J. (1992). Disease development in ethylene-insensitive *Arabidopsis thaliana* infected with virulent and avirulent *Pseudomonas* and *Xanthomonas* pathogens. Mol. Plant-Microbe Interact. **5**, 372–378.

Carland, F.M., and Staskawicz, B.J. (1993). Genetic characterization of the *Pto* locus of tomato: Semi-dominance and cosegregation of resistance to *Pseudomonas syringae* pathovar tomato and sensitivity to the insecticide Fenthion. Mol. Gen. Genet. **239**, 17–27.

Chang, C., Bowman, J.L., De John, A.W., Lander, E.S., and Meyerowitz, E.M. (1988). Restriction fragment length polymorphism linkage map for *Arabidopsis thaliana*. Proc. Natl. Acad. Sci. USA **85**, 6856–6860.

Cuppels, D.A. (1986). Generation and characterization of Tn5 insertion mutations in *Pseudomonas syringae* pv. *tomato*. Appl. Environ. Microbiol. **52**, 323–327.

Dangl, J.L. (1992). Applications of *Arabidopsis thaliana* to outstanding issues in plant-pathogen interactions. Int. Rev. Cytol., **144**, 53–83.

Dangl, J.L., Ritter, C., Gibbon, M.J., Mur, L.A.J., Wood, J.R., Goss, S., Mansfield, J., Taylor, J.D., and Vivian, A. (1992). Functional homologs of the Arabidopsis *RPM1* disease resistance gene in bean and pea. Plant Cell, **4**, 1359–1369.

Davis, K.R., Schott, E., and Ausubel, F.M. (1991). Virulence of selected phytopathogenic pseudomonads in *Arabidopsis thaliana*. Mol. Plant-Microbe Interact. **4**, 477–488.

Debener, T., Lehnackers, H., Arnold, M., and Dangl, J. (1991). Identification and molecular mapping of a single *Arabidopsis thaliana* locus determining resistance to a phytopathogenic *Pseudomonas syringae* isolate. Plant J. **1**, 289–302.

Dong, X., Mindrinos, M., Davis, K.R., and Ausubel, F.M. (1991). Induction of *Arabidopsis* defense genes by virulent and avirulent *Pseudomonas syringae* strains and by a cloned avirulence gene. Plant Cell **3,** 61–72.

Figurski, D., and Helinski, D.R. (1979). Replication of an origin-containing derivative of plasmid RK2 is dependent on a plasmid function provided in *trans*. Proc. Natl. Acad. Sci. USA **76,** 1648–1652.

Flor, H. (1971). Current status of the gene-for-gene concept. Annu. Rev. Phytopathol. **9,** 275–296.

Gabriel, D.W., and Rolfe, B.G. (1990). Working models of specific recognition in plant-microbe interactions. Annu. Rev. Phytopathol. **28,** 365–391.

Gamborg, O.L., Miller, R.A., and Ojima, K. (1968). Nutrient requirements of suspension cultures of soybean root cells. Exp. Cell Res. **50,** 151–158.

Innes, R.W., Bent, A.F., Kunkel, B.N., Bisgrove, S.R. and Staskawicz, B.J. (1993a). Molecular analysis of avirulence gene *avrRpt2* and identification of a putative regulatory sequence common to all known *Pseudomonas syringae* avirulence genes. J. Bacteriol. **175,** in press.

Innes, R.W., Bisgrove, S.R., Smith, N.M., Bent, A.F., Staskawicz, B.J., and Liu, Y. (1993b). Identification of a disease resistance locus in Arabidopsis that is functionally homologous to the RPG1 locus of soybean. Plant J., in press.

Johal, G.S., and Briggs, S.P. (1992). Reductase activity encoded by the *HM1* disease resistance gene in maize. Science **258,** 985–987.

Jorgensen, J.H. (1988). Genetic analysis of barley mutants with modifications of powdery mildew resistance gene *Ml-a12*. Genome **30,** 129–132.

Keen, N.T. (1990). Gene-for-gene complementarity in plant-pathogen interactions. Annu. Rev. Genet. **24,** 447–463.

Keen, N.T., Tamaki, S., Kobayashi, D., Gerhold, D., Stayton, M., Shen, H., Gold, S., Lorang, J., Thordal-Christensen, H., Dahlbeck, D., and Staskawicz, B. (1990). Bacteria expressing avirulence gene D produce a specific elicitor of the soybean hypersensitive reaction. Mol. Plant-Microbe Interact. **3,** 112–121.

Klement, Z. (1982). Hypersensitivity. In Phytopathogenic Procaryotes, M.S. Mount and G.H. Lacy eds (New York: Academic Press), pp. 149–177.

Koch, E., and Slusarenko, A. (1990). *Arabidopsis* is susceptible to infection by a downy mildew fungus. Plant Cell, **2** 437–445.

Koornneef, M., Dellaert, L.W.M., and van der Veen, J.H. (1982). EMS- and radiation-induced mutation frequencies at individual loci in *Arabidopsis thaliana* (L.) Heynh. Mutat. Res. **93,** 109–123.

Kosambi, D.D. (1944). The estimation of map distances from recombination values. Ann. Eugen. **12,** 172–175.

Lamb, C.J., Lawton, M.A., Dron, M., and Dixon, R.A. (1989). Signals and transduction mechanisms for activation of plant defenses against microbial attack. Cell **56,** 215–224.

Lander, E.S., Green, P., Abrahamson, J., Barlow, A., Daly, M.J., Lincoln, S.E., and Newburg, L. (1987). Mapmaker: An interactive computer package for constructing primary genetic linkage maps of experimental and natural populations. Genomics **1,** 174–181.

Lindgren, P.B., Peet, R.C., and Panopoulos, N.J. (1986). Gene cluster of *Pseudomonas syringae* pv. *"phaseolicola"* controls pathogenicity of bean plants and hypersensitivity on nonhost plants. J. Bacteriol. **168,** 512–522.

Maniatis, T., Fritsch, E.F., and Sambrook, J. (1989). Molecular Cloning: A Laboratory Manual, 2nd Ed. (Cold Spring Harbor, NY: Cold Spring Harbor Laboratory).

Meeley, R.B., Johal, G.S., Briggs, S.P., and Walton, J. D. (1992). A biochemical phenotype for a disease resistance gene of maize. Plant Cell **4,** 71–77.

Nam, H.-G., Giraudat, J., den Boer, B., Moonan, F., Loos, W. D.B., Hauge, B.M., and Goodman, H.M. (1989). Restriction fragment length polymorphism linkage map of *Arabidopsis thaliana*. Plant Cell **1,** 699–705.

Schroth, M.N., Hildebrand, D.C., and Starr, M.P. (1981). Phytopathogenic members of the genus *Pseudomonas*. In The Prokaryotes, M.P. Starr, H. Stolp, H.G. Trüper, A. Balows, and H. G. Schlegel, eds (Berlin: Springer-Verlag), pp. 701–718.

Simon, A.E., Li, X. H., Lew, J.E., Stange, R., Zhang, C., Polacco, M., and Carpenter, C.D. (1992). Susceptibility and resistance of *Arabidopsis thaliana* to turnip crinkle virus. Mol. Plant-Microbe Interact. **5,** 496–503.

Simpson, R.B., and Johnson, L.J. (1990). *Arabidopsis thaliana* as a host for *Xanthomonas campestris* pv. *campestris*. Mol. Plant-Microbe Interact. **3,** 233–237.

Staskawicz, B., Dahlbeck, D., Keen, N., and Napoli, C. (1987). Molecular characterization of cloned avirulence genes from race 0 and race 1 of *Pseudomonas syringae* pv. *glycinea*. J. Bacteriol. **169,** 5789–5794.

Tai, T.H., and Tanksley, S.D. (1990). A rapid and inexpensive method for isolation of total DNA from dehydrated plant tissue. Plant Mol. Biol. Rep. **8,** 297–303.

Tamaki, S., Dahlbeck, D., Staskawicz, B., and Keen, N.T. (1988). Characterization and expression of two avirulence genes cloned from *Pseudomonas syringae* pv. *glycinea*. J. Bacteriol. **170,** 4846–4854.

Torp, J., and Jorgensen, J.H. (1986). Modification of barley powdery mildew resistance gene *Ml-a12* by induced mutation. Can. J. Genet. Cytol. **28,** 725–731.

Tsuji, J., Somerville, S.C., and Hammerschmidt, R. (1991). Identification of a gene in *Arabidopsis thaliana* that controls resistance to *Xanthomonas campestris* pv. *campestris*. Physiol. Mol. Plant Pathol. **38,** 57–65.

Van den Ackerveken, G.F.J.M., Van Kan, J.A.L. and De Wit, P. J.G.M. (1992). Molecular analysis of the avirulence gene avr9 of the fungal tomato pathogen *Cladosporium fulvum* fully supports the gene-for-gene hypothesis. Plant J. **2,** 359–366.

Whalen, M.C., Stall, R.E., and Staskawicz, B.J. (1988). Characterization of a gene from a tomato pathogen determining hypersensitive resistance in non-host species and genetic analysis of this resistance in bean. Proc. Natl. Acad. Sci. USA **85,** 6743–6747.

Whalen, M.C., Innes, R.W., Bent, A.F., and Staskawicz, B.J. (1991). Identification of *Pseudomonas syringae* pathogens of *Arabidopsis* and a bacterial locus determining avirulence on both *Arabidopsis* and soybean. Plant Cell **3,** 49–59.

Williams, J.G., Kubelik, A.R., Livak, K.J., Rafalski, J.A., and Tingey, S.V. (1990). DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. Nucl. Acids Res. **18,** 6531–6535.

Yu, G., Katagiri, K., and Ausubel, F.M. (1993). *Arabidopsis* mutations at the *RPS2* locus result in loss of resistance to *Pseudomonas syringae* strains expressing the avirulence gene *avrRpt2*. Mol. Plant-Microbe. Interact, in press.