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

Barbara N. Kunkel,*1 Andrew F. Bent,*1 Douglas Dahlbeck,*1 Roger W. Innes,*b2 and Brian J. Staskawicz*a9,3

*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 Wu-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 *HM7*, 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 *HM7* 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 (Schröth 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;
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.

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[avrRmp1]. 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 M2 plants derived from seed of the resistant ecotype Col-0 that had been mutagenized with diepoxybutane. Of ~7500 M2 plants tested, four plants were found to be susceptible to Pst DC3000[avrRpt2] upon retesting in the M3 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 M3 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 105 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-O and No4 Inoculated with Several Pst Strains

<table>
<thead>
<tr>
<th>Pst</th>
<th>Arabidopsis</th>
<th>rps2-201</th>
<th>Col-0 x rps2-201 F₁</th>
<th>Wü-O</th>
<th>No4</th>
</tr>
</thead>
<tbody>
<tr>
<td>DC3000</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td></td>
<td>4.3 ± 0.07</td>
<td>4.2 ± 0.10</td>
<td>4.0 ± 0.36</td>
<td>4.3 ± 0.13</td>
<td>3.9 ± 0.20</td>
</tr>
<tr>
<td></td>
<td>n = 191</td>
<td>n = 111</td>
<td>n = 15</td>
<td>n = 34</td>
<td>n = 34</td>
</tr>
<tr>
<td>DC3000(avrRpt2)</td>
<td>R</td>
<td>S</td>
<td>S</td>
<td>S</td>
<td>R</td>
</tr>
<tr>
<td></td>
<td>1.8 ± 0.10</td>
<td>4.3 ± 0.09</td>
<td>3.8 ± 0.36</td>
<td>3.2 ± 0.18</td>
<td>2.0 ± 0.27</td>
</tr>
<tr>
<td></td>
<td>n = 126</td>
<td>n = 114</td>
<td>n = 17</td>
<td>n = 37</td>
<td>n = 26</td>
</tr>
<tr>
<td>DC3000(avrB)</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td>R</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2.0 ± 0.17</td>
<td>1.9 ± 0.23</td>
<td>1.2 ± 0.2</td>
<td>1.2 ± 0.14</td>
<td>nt</td>
</tr>
<tr>
<td></td>
<td>n = 64</td>
<td>n = 29</td>
<td>n = 5</td>
<td>n = 16</td>
<td></td>
</tr>
<tr>
<td>DC3000(avrRpm1)</td>
<td>R</td>
<td>R</td>
<td>nt</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>1.3 ± 0.16</td>
<td>1.6 ± 0.33</td>
<td>nt</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>n = 15</td>
<td>n = 14</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Disease symptoms were scored 5 days after pipette infiltration with bacteria at 10⁶ 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.

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 10⁵ to 10⁶ cfu/cm² 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-O plants, which obtained a final concentration of only 10⁴ to 10⁵ cfu/cm² (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 expressingavrRpt2. As summarized in Table 2 and Figure 3, wild-type Col-0 plants inoculated with high levels of P. syringae strains expressingavrRpt2 exhibited tissue collapse and necrosis in the inoculated region of the leaf within 24 hr after infiltration. Inoculation with P. syringae strains lackingavrRpt2 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 expressingavrRpt2 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...
Table 2. HR of Susceptible Mutant rps2-201 and Ecotypes Wü-O and No-0a

<table>
<thead>
<tr>
<th>Arabidopsis</th>
<th>Col-0 (wt)</th>
<th>rps2-201</th>
<th>Col-0 × rps2-201 F1</th>
<th>Wü-O</th>
<th>No-O</th>
</tr>
</thead>
<tbody>
<tr>
<td>P. syringaeb</td>
<td>No HR</td>
<td>No HR</td>
<td>No HR</td>
<td>No HR</td>
<td>No HR</td>
</tr>
<tr>
<td>P. syringae (avrRpt2)</td>
<td>H1.0 ± 0.01</td>
<td>H1.1 ± 0.04</td>
<td>H1.2 ± 0.11</td>
<td>H1.0 ± 0.04</td>
<td>H1.0 ± 0.03</td>
</tr>
<tr>
<td>n = 66</td>
<td>n = 72</td>
<td>n = 21</td>
<td>n = 52</td>
<td>n = 16</td>
<td></td>
</tr>
<tr>
<td>P. syringae (avrB)</td>
<td>HR</td>
<td>No HR</td>
<td>HR</td>
<td>No HR</td>
<td>HR</td>
</tr>
<tr>
<td>P. syringae (avrRpm1)</td>
<td>HR</td>
<td>HR</td>
<td>HR</td>
<td>HR</td>
<td>HR</td>
</tr>
<tr>
<td>n = 94</td>
<td>n = 132</td>
<td>n = 26</td>
<td>n = 69</td>
<td>n = 28</td>
<td></td>
</tr>
<tr>
<td>P. syringae (avrRpm1)</td>
<td>HR</td>
<td>HR</td>
<td>nt</td>
<td>H1.9 ± 0.18</td>
<td>nt</td>
</tr>
<tr>
<td>n = 26</td>
<td>n = 13</td>
<td>nt</td>
<td>n = 29</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P. syringae (avrRpm1)</td>
<td>HR</td>
<td>HR</td>
<td>H2.5 ± 0.22</td>
<td>nt</td>
<td>nt</td>
</tr>
<tr>
<td>n = 26</td>
<td>n = 13</td>
<td>nt</td>
<td>n = 29</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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 pLH12Q (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, Noszen (No-0; Table 1). The F1 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 F1 progeny were susceptible to Pst DC3000(avrRpt2) (Table 1; data not shown for rps2-201 × No-0 F1). When challenged with a lower dose of inoculum (i.e., 10^5 cfu/mL), F1 plants exhibited no disease symptoms, whereas

The HR in Col-0 (wt) (●), rps2-201 × Col-0 F1 (▲), and rps2-201 (■) plants was scored at the indicated times after pipette infiltration with 10^6 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 F₁ 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 the F₁ 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 F₁ progeny did not exhibit strong tissue collapse until 40 to 48 hr after inoculation (Figure 3). The intermediate resistance phenotype of the F₁ 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 F₂ 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 F₂ 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 F₂ individuals from the Col-0 *gfit* × *rps2-201* cross and 140 F₂ individuals from the *rps2-201* × No-0 cross. As expected, the F₃ 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 F₁ progeny from these crosses were intermediate in their resistance phenotype.

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

<table>
<thead>
<tr>
<th>Cross</th>
<th>Generation</th>
<th>Number of Plants</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Resistant</td>
<td>Susceptible</td>
<td>Total</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>rps2-201/rps2-201 × RPS2/RPS2 (Col-0) F₁</td>
<td>5</td>
<td>0</td>
<td>5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>× RPS2/RPS2 (Col-0) F₂</td>
<td>86</td>
<td>24</td>
<td>110</td>
<td>0.59</td>
<td>0.44</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RPS2 <em>gfit</em>/*RPS2 <em>gfit</em> × rps2-201/rps2-201 F₁</td>
<td>5</td>
<td>0</td>
<td>5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>× rps2-201/rps2-201 F₂</td>
<td>97</td>
<td>43</td>
<td>140</td>
<td>2.44</td>
<td>0.12</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>rps2-201/rps2-201 × RPS2/RPS2 (No-0) F₁</td>
<td>5</td>
<td>0</td>
<td>5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>× RPS2/RPS2 (No-0) F₂</td>
<td>227</td>
<td>65</td>
<td>294</td>
<td>1.17</td>
<td>0.28</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

| Deduced F₃ Genotypes | R/R | R/S | S/S | Total | | | | | |
| --- | --- | --- | --- | --- | | | | | |
| RPS2 *gfit*/*RPS2 *gfit* × rps2-201/rps2-201 F₃ families | 16 | 39 | 18 | 73 | 0.45 | 0.80 | | | |
| rps2-201/rps2-201 × RPS2/RPS2 (No-0) F₃ families | 33 | 68 | 39 | 140 | 0.63 | 0.73 | | | |

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

*b* $\chi^2$ values were calculated for a segregation ratio of 3 resistant:1 susceptible plant.

*c* F₃ families were obtained by allowing individual F₂ plants to self-fertilize. For progeny testing, a minimum of 12 individuals per F₃ family were tested.

*d* $\chi^2$ 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 F2 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 F1 and F2 progeny for resistance to *Pst* strain DC3000(*avrRpt2*) by the L-77 inoculation procedure. The lack of complementation in the F1 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 F2 progeny from these crosses to map the additional rps mutations with respect to rps2-201. The absence of resistant plants among the F2 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 F2 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*

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

<table>
<thead>
<tr>
<th>Cross</th>
<th>Number of Plants</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Generation</td>
</tr>
<tr>
<td>RPS2 gl1/RPS2 gl1^c^</td>
<td>F1</td>
</tr>
<tr>
<td>(\times) rps2-202/rps2-202</td>
<td></td>
</tr>
<tr>
<td>rps2-201 gl1/rps2-201 gl1</td>
<td>F1</td>
</tr>
<tr>
<td>(\times) rps2-202/rps2-202</td>
<td>F2</td>
</tr>
<tr>
<td>RPS2 gl1/RPS2 gl1</td>
<td>F1</td>
</tr>
<tr>
<td>(\times) rps2-203/rps2-203</td>
<td>F2</td>
</tr>
<tr>
<td>rps2-201 gl1/rps2-201 gl1</td>
<td>F1</td>
</tr>
<tr>
<td>(\times) rps2-203/rps2-203</td>
<td>F2</td>
</tr>
<tr>
<td>RPS2/RPS2</td>
<td>F1</td>
</tr>
<tr>
<td>(\times) rps2-301/rps2-301</td>
<td>F2</td>
</tr>
<tr>
<td>rps2-201 gl1/rps2-201</td>
<td>F1</td>
</tr>
<tr>
<td>(\times) rps2-301/rps2-301</td>
<td>F2</td>
</tr>
<tr>
<td>rps2-201 gl1/rps2-201 gl1</td>
<td>F2</td>
</tr>
<tr>
<td>(\times) rps2-101/rps2-101</td>
<td></td>
</tr>
<tr>
<td>RPS2/RPS2</td>
<td>F1</td>
</tr>
<tr>
<td>(\times) rps2-204/rps2-204</td>
<td>F2</td>
</tr>
<tr>
<td>(Wü-0)</td>
<td></td>
</tr>
<tr>
<td>rps2-201 gl1/rps2-201</td>
<td>F1</td>
</tr>
<tr>
<td>(\times) rps2-204/rps2-204</td>
<td>F2</td>
</tr>
</tbody>
</table>

^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^ \(\chi^2\) 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 F1 progeny were wild type for trichome development and the F2 progeny segregated in a ratio of 3 wild type:1 glabrous.

^d^ Segregation of resistance among the F2 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.

^e^ F2 progeny from these F2 individuals were not available for analysis. However, in 12 of 12 other cases in which putatively resistant plants from predominantly susceptible F2 populations were subjected to progeny analysis, the resistant F2 plants gave rise to F3 families that segregated 100% for susceptible plants or were determined to be seed contaminants (see Methods).
DC3000(avnRpt2) 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 F2 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-O

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-O), that is susceptible to Pst DC3000(avnRpt2) (Figure 1). Wü-0 plants inoculated with Pst DC3000(avnRpt2) by pipette infiltration with 10^6 cfu/mL Pst DC3000(avnRpt2) developed extensive, gray-brown necrotic lesions within 5 days after inoculation (Table 1). However, the disease symptoms produced by Pst DC3000(avnRpt2) on ecotype Wü-O 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(avnRpt2) 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-O 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 F1 progeny were resistant to Pst DC3000(avnRpt2) (Table 4). Resistance in the F2 progeny from this cross segregated as a monogenic trait (Table 4). To determine whether susceptibility to Pst DC3000(avnRpt2) 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 F2 progeny from this cross, we observed 269 susceptible individuals from a total of 274 F2 plants scored (Table 4), suggesting that susceptibility in ecotype Wü-O 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 x No-0 cross, 116 F3 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.](image-url)
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 F2 progeny of the rps2-201 × No-0 crosses. We identified three recombinants between RN37 and RPS2 from 70 chromosomes scored, placing RPS2 at approximately 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.

F1 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. F1 plants inoculated by the L-77 dipping procedure were phenotypically resistant (Table 3), whereas the resistance phenotype of F1 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 F1, and F2 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 F2 progeny. The absence of resistant plants among the F2 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 F2 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, Zu-0 (R. Innes, unpublished results), and Wü-O (Figure 1; Table 2), which are susceptible to Pst DC3000 (avrRpt2). However, disease symptoms produced on Wü-O 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-O carries a partially functional allele of RPS2. Wü-O, like the rps2-201 mutants, retained resistance to Pst strains expressing other avirulence genes. Additionally, genetic analysis indicated that susceptibility in Wü-O 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, ~2.5 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, 1986) reported the isolation of 25 mutants with altered resistance to powdery mildew, 23 of which mapped to the Mf-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, 1988) 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 pVS88 by triparental mating using the helper plasmid pRK2013 (Figurski and Helinski, 1979). Plasmid pVS88 carries the *avrRpt2* gene from *Pst* strain 1056 and was constructed by ligation of a 1.5-kb SalI fragment from pVSP61 (Whalen et al., 1991) or pVS88 by triparental mating using the helper plasmid pRK2013. Plasmid pVS61, pVS61 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 pVS61. Strains expressing *avrRpm1* were constructed by the introduction of plasmid K48 by triparental mating (Debener et al., 1991). *P. syringae* strains not expressing cloned avrulence genes carried control plasmids pLAFR3 (vector without insert), pLH120 (an insertionally inactivated derivative of pLH12; Whalen et al., 1991), or pVS61.

**Plant Material, Growth Conditions, and Inoculation Procedures**

Arabidopsis ecotypes Colomba (Col-0), Nossen (Nc-0), and Würzburg (Wü-0) were obtained from the Arabidopsis Information Service Seed Bank. Col-0 gfr 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 μE m⁻² sec⁻¹. Entire leaf rosettes of 5-week-old plants were dipped into bacterial suspensions of 2 to 3 × 10⁶ colony-forming units (cfu)/ml in 10 mM MgCl₂ 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 MgCl₂ to an OD₆₀₀ of 0.001 (~10⁶ 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 MgCl₂ to an *OD*₆₀₀ of 0.02 (~2 × 10⁷ cfu/ml) for *Pst* DC3000 strains and to an OD₆₀₀ of 0.1 (~10⁸ cfu/ml) for *Psp* 3121 strains. Leaves were scored for tissue collapse ~24 hr after inoculation and again at 48 hr after inoculation. Leaves infiltrated with *Psp* strain 3121 not expressing cloned avrulence 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 M₁ seeds were planted separately to obtain independent populations of mutagenized *M₂* 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 *M₃* 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 *M₃* generation. True-breeding, susceptible *rps2-202* and *rps2-203* lines used for further analysis were obtained from susceptible *M₃* 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 gfr, a marked line that lacks leaf and stem trichomes (glabrous; Koornneef et al., 1982). In crosses where the female parent carried the gfr mutation, all *F₁* progeny had normal trichomes, and the *F₂* progeny segregated in a ratio of 3 wild-type:1 glabrous plant. *F₃* progeny from crosses between *rps2-201* and
ecotypes No-0 and Wi-0 were verified as being true cross progeny
by DNA gel blot analysis, using restriction fragment length polymor-
phism (RFLP) markers that reveal polymorphisms between the different
ecotypes as probes. Allelism tests were performed by crossing the
rps mutants to a marked rps2-207 line carrying the glf mutation, ex-
cept for mutant rps2-307, which was crossed to an unmarked rps2-201
line. Individual F2 progeny were scored for their resistance phenotype
by surfactant inoculation with Pst DC3000(avrRpt2). F2 families were
obtained by allowing individual F1 plants to self-fertilize. For progeny
testing, a minimum of 12 individuals per F2 family were tested for their
resistance phenotype using the surfactant inoculation procedure. F2
plants that gave rise to F2 families consisting of only resistant indi-
viduals were scored as homozygous resistant (RPS2/RPS2), F2 plants
that gave rise to F2 families consisting of both resistant and suscep-
tible individuals were scored as heterozygous (RPS2/rps2), and F2
plants that gave rise to F2 families consisting of only susceptible indi-
viduals were scored as homozygous susceptible (rps2/rps2). Among
the putative resistant F2 progeny derived from the cross of rps2-201
× rps2-107 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 were isolated from 115
F2 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 per-
formed 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 func-
tion (Kosambi, 1944). Plant DNA was isolated according to the method
of Tai and Tankersley (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, GGGCG-
GTAA and p37, GGTACCAGAG. Polymerase chain reaction (PCR)
reactions contained 50 mM KCl, 10 mM Tris-HCl, pH 8.3, 2 mM MgCl2,
0.001% gelatin, 3% DMSO, 100 pM each of 4 dNTPs, 200 pM primer,
10 ng genomic DNA, and 1 unit of Taq Polymerase (Perkin-Elmer Ce-
tus) in a 20-μL volume overlaid with 40 μL mineral oil. Amplification
was performed in a thermal cycler (model PHC2; Techne, Inc., Prince-
ton, NJ) or a thermal controller (MJ Research, Watertown, MA)
programmed for 10 cycles of 1 min at 94℃, 1 min at 35℃, 15 sec at
45℃, and 1 min, 45 sec at 72℃, followed by 35 cycles of 1 min at
92℃, 1 min at 35℃, 15 sec at 45℃, and 1 min, 45 sec at 72℃. Am-
plication 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
the resistant ecotype No-0. The DNAs tested were isolated from 115
individuals scored for resistance to the insecticide Fenthion. MOI. Gen.
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