Characterization of eds1, a Mutation in Arabidopsis Suppressing Resistance to Peronospora parasitica Specified by Several Different RPP Genes

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The interaction between Arabidopsis and the biotrophic oomycete Peronospora parasitica (downy mildew) provides an attractive model pathosystem to identify molecular components of the host that are required for genotype-specific recognition of the parasite. These components are the so-called RPP genes (for Resistance to P. parasitica). Mutational analysis of the ecotype Wassilewskija (Ws-O) revealed an RPP-nonspecific locus called EDS1 (for enhanced disease susceptibility) that is required for the function of RPP genes on chromosomes 3 (RPP1/RPP14 and RPP10) and 4 (RPP12). Genetic analyses demonstrated that the eds1 mutation is recessive and is not a defective allele of any known RPP gene, mapping to the bottom arm of chromosome 3 (~13 centimorgans below RPP1/RPP14). Phenotypically, the Ws-edsl mutant seedlings supported heavy sporulation by P. parasitica isolates that are each diagnostic for one of the RPP genes in wild-type Ws-O; none of the isolates is capable of sporulating on wild-type Ws-O. Ws-edsl seedlings exhibited enhanced susceptibility to some P. parasitica isolates when compared with a compatible wild-type ecotype, Columbia, and the eds1 parental ecotype, Ws-O. This was observed as earlier initiation of sporulation and elevated production of conidiophores. Surprisingly, cotyledons of Ws-edsl also supported low sporulation by five isolates of P. parasitica from Brassica oleracea. These isolates were unable to sporulate on >100 ecotypes of Arabidopsis, including wild-type Ws-0. An isolate of Albugo candida (white blister) from B. oleracea also sporulated on Ws-edsl, but the mutant exhibited no alteration in phenotype when inoculated with several oomycete isolates from other host species. The bacterial resistance gene RPM1, conferring specific recognition of the avirulence gene avrB from Pseudomonas syringae pv glycinea, was not compromised in Ws-edsl plants. The mutant also retained full responsiveness to the chemical inducer of systemic acquired resistance, 2,6-dichloroisonicotinic acid; Ws-edsl seedlings treated with 2,6-dichloroisonicotinic acid became resistant to the Ws-O-compatible and Ws-O-incompatible P. parasitica isolates Emwal and Noc02, respectively. In summary, the EDS1 gene appears to be a necessary component of the resistance response specified by several RPP genes and is likely to function upstream from the convergence of disease resistance pathways in Arabidopsis.

INTRODUCTION

Disease resistance in plants to microbial pathogens is often triggered by recognition of the invading pathogen by the host in a race-specific manner (Crute, 1985; Keen, 1990; Pryor and Ellis, 1993). In many cases, this is accompanied by localized host cell death at the site of infection (the so-called hypersensitive response [HR]) and a series of defense-related reactions, such as an oxidative burst, cell wall reinforcements, and the accumulation of pathogenesis-related proteins (Lamb, 1994). Although there is a strong correlation between the induction of these responses and the occurrence of disease resistance, their actual role in limiting pathogen growth is not clear.

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A key objective in plant pathology is to unravel the molecular mechanisms underlying recognition specificity and the associated signaling events leading to disease resistance or susceptibility. The cloning of several race-specific disease resistance (R) genes from different dicotyledonous species provided some clues about their possible function and localization in the plant cell (Staskiwicz et al., 1995). Their deduced amino acid sequences revealed the presence of either leucine-rich repeat (LRR) and a serine-threonine kinase domain (Martin et al., 1993; Zhou et al., 1995), implicating protein phosphorylation as a key feature of the pathogen recognition process.
These possible roles have been reinforced by sequence analysis of the rice Xa21 gene for race-specific resistance to *Xanthomonas oryzae* (Song et al., 1995). Xa21 possesses predicted extracellular leucine-rich repeats, a membrane-spanning domain, and an intracellular portion containing a serine/threonine kinase motif. Therefore, this molecule appears to exhibit a combined role in both cell surface recognition of the pathogen and in activation of an intracellular defense signal.

The striking similarities in the predicted structural motifs among the cloned R genes suggest that resistance in different dicotyledonous and monocotyledonous species to a diverse range of microbial pathogens may operate, at least in part, by similar mechanisms. However, the precise course of biochemical events and the number of other components required for a particular R gene-mediated signaling pathway are not yet known.

In recent years, Arabidopsis (mouse-ear cress) has been studied intensively as a model host plant for diseases caused by bacterial, fungal, and viral pathogens and provides a genetically amenable system to examine the various components of disease resistance (Dangl, 1993; Kunkel, 1996). Two R genes specifying race-specific resistance to *Pseudomonas syringae* strains were isolated using a positional cloning strategy (Bent et al., 1994; Mindrinos et al., 1994; Grant et al., 1995). Also, mutational screens for plants altered in resistance have led to the identification of defective mutant alleles of these respective R genes (Kunkel et al., 1993; Yu et al., 1993; Bisgrove et al., 1994) as well as a mutation in an additional gene, *NDR1* (for non-specific disease resistance), encoding a potential signaling component required for the function of several different race-specific R genes (Century et al., 1995). Components of systemically induced resistance responses (Bowling et al., 1994; Cao et al., 1994; Delaney et al., 1995) and other potential defense pathways (Dietrich et al., 1994; Glazebrook and Ausubel, 1994; Greenberg et al., 1994; Ausubel et al., 1995) have also been characterized in Arabidopsis.

The interaction between Arabidopsis and the biotrophic oomycete *Peronospora parasitica* (downy mildew) provides an attractive system for the genetic dissection of pathways determining recognition specificity in race-specific resistance and the proper manifestation of the resistance response (Crute et al., 1993, 1994a, 1994b; Holub and Beynon, 1996). *P. parasitica* is a parasite exclusive to members of the Cruciferae (Channon, 1981). Natural *P. parasitica* isolates have been identified that are able to complete their life cycle on certain ecotypes of Arabidopsis by producing asexual and sexual spores (Koch and Siusarenko, 1990; Crute et al., 1993). Additional genetic studies by Holub et al. (1994) demonstrated a large natural variation among Arabidopsis ecotypes in their interaction phenotypes with particular isolates of the parasite. This is characteristic of gene-for-gene relationships (Fior, 1971), and the existence of at least 25 different RPP (for resistance to *P. parasitica*) genes (known also as recognition specificities) has been predicted from segregation analyses (Parker et al., 1993; Crute et al., 1994a, 1994b; Holub et al., 1994; Tör et al., 1994; Holub and Beynon, 1996; Reignault et al., 1996).

Two RPP loci have been analyzed in detail with respect to recognition of the Noco2 isolate of *P. parasitica*. RPP5 was identified in the ecotype Landsberg erecta (Ler-0) and was mapped to the upper arm of chromosome 4 (Parker et al., 1993). A second locus, RPP14, in the ecotype Wassilewskija (Ws-0) maps to a cluster of several other RPP genes on the lower arm of chromosome 3 (Reignault et al., 1996). It has not yet been separated by recombination from RPP1 or RPP10, associated with recognition of isolates Emoy2 and Cala2, respectively (Holub et al., 1994; Holub and Beynon, 1996). The RPP gene-mediated resistance responses are characterized by hypersensitive plant cell necrosis initiated in the mesophyll at sites of attempted mycelium ingress. Fungal development beyond these lesions is severely restricted. In contrast, ecotype Columbia (Col-0) is susceptible to Noco2, and its mycelium is able to spread systemically through the plant tissue and to develop asexual and sexual spores on inoculated tissue within 4 to 7 days.

A mutational approach was adopted to identify genes that are necessary for resistance mediated by RPP5 and RPP14 and to attempt separation of RPP14 from the other closely linked RPP gene specificities. Therefore, we screened mutagenized populations of Ler-0 and Ws-0 for mutations that caused a change from Noco2 resistance to susceptibility. Here, we describe a recessive mutation of Ws-0 called eds1 (for enhanced disease susceptibility), which abolishes the resistance mediated by RPP14 as well as by other linked and unlinked RPP genes present in the Ws-0 background. This mutation also partially suppresses resistance of Ws-0 to five *Brassica oleracea*–infecting isolates of *P. parasitica* to which all Arabidopsis ecotypes so far tested exhibit resistance, implicating a possible common functional role for the EDS1 protein in downy mildew resistance in Arabidopsis and Brassica plants. The potential role of EDS1 in resistance mediated by RPP genes and in the context of other disease resistance signaling pathways is discussed.

**RESULTS**

**Identification of Mutations in Ws-0 for Susceptibility to *P. parasitica* Isolate Noco2**

The cotyledons of wild-type Ws-0 seedlings responded after inoculation with the *P. parasitica* isolate Noco2 by producing distinct necrotic lesions beginning 3 to 4 days after inoculation (DAI), as shown in Figure 1A. The interaction phenotype between Ws-0 and Noco2 has been described at a later stage (7 DAI) as "a pitting necrosis with no parasite sporulation" (Holub et al., 1994) and is associated with a single recognition locus, RPP14 (Reignault et al., 1996). This locus cosegregates with RPP1 and RPP10, which specify resistance in Ws-0 to isolates Emoy2 and Cala2, respectively (Holub et al., 1994).
separation of RPP14 from RPP1 and RPP10 in Ws-0. To this end, the isolate Noco2 was used to screen for Noco2-compatible mutations of Ws-0 that were altered solely in response to the selective isolate. Approximately 30,000 M2 seedlings germinated from ethyl methanesulfonate-mutagenized Ws-0 seed were spray inoculated with Noco2; this included 38 M2 seed populations that were each derived independently from pools of ~50 M1 plants. Six independently mutated M2 plants were selected, and the first of these (EW80-1) is described here. Characterization of RPP14-specific mutants will be presented elsewhere.

Ten cleaved amplified polymorphic DNA sequence (CAPS; Konieczny and Ausubel, 1993) or microsatellite (Bell and Ecker, 1994) markers representing the five Arabidopsis chromosomes were used to confirm that EW80-1 was in fact a mutation of Ws-0 and not a contaminant derived from outcross pollination. The DNA profiles of EW80-1 were identical to those of wild-type Ws-0 with each marker and were distinguished from the two Noco2-susceptible ecotypes COLO and Niederzenz (Nd-O) (results not shown). It was therefore highly unlikely that EW80-1 arose from a contaminant seed. M2 progeny were all susceptible to Noco2, and a single M2 plant was selected for subsequent genetic and phenotypic analyses.

Genetic Analyses and Mapping of the eds1 Mutation in EW80-1

Crossovers were made between EW80-1 and the wild-type parent Ws-0 (containing RPP14) and with the susceptible ecotype Col-5 (Col-0 containing gl/1, a recessive phenotypic marker for a glabrous mutation). The segregation of resistance and susceptibility was then analyzed in F1, and F2 progeny generated from the two crossovers, and the results are shown in Table 1.

In the cross between EW80-1 and Ws-0, all F1 seedlings were resistant to Noco2, indicating that the mutation is recessive. This was confirmed by microscopic examination of the inoculated seedlings after staining with lactophenol trypan blue, which preferentially stains fungal structures as well as dead or dying plant cells (Keogh et al., 1980). The results indicated that the degree of plant cell necrosis and fungal development was similar in F1 and wild-type Ws-0 seedlings (data not shown). Analysis of F2 EW80-1 x Ws-0 progeny confirmed that EW80-1 contains a single recessive mutation because there was a 3:1 segregation of resistance to susceptibility.

The mutation that enhances disease susceptibility in EW80-1 is referred to hereafter as eds1, based on evidence from the phenotypic analyses described below. The Ws-0 mutant line EW80-1 is accordingly named Ws-eds1. eds has also been used to name several newly identified mutante loci that confer enhanced susceptibility in Col-0 to a mildly virulent strain of the bacterial pathogen P. syringae pv maculicola (Glazebrook et al., 1996; see also Discussion).

In the cross between Ws-eds1 (EW80-1) and Col-5, all F1 seedlings were also phenotypically resistant to Noco2 (Table 1), indicating that the mutation was complemented by a gene in Col-5 and was therefore not an allele of RPP14. Segregation analysis of F2 progeny for this cross (Table 1) showed that the ratio of resistant-to-susceptible plants is consistent with the presence of a single recessive mutation in Ws-0 at a second locus required for RPP14 function. Linkage of the mutation with RPP14 was inconclusive from these genetic data.

Molecular markers and the glabrous phenotype of gl/1 were used to determine a map location for the eds1 mutation. Approximately 130 F2 progeny from the Col-5 x EW80-1 cross, which were segregating for both RPP14 and EDS1, were selected for full Noco2 susceptibility. The selected plants were then further analyzed to determine the presence of the RPP14 gene from Ws-0 by using closely linked molecular markers. These markers were vnt21, lying 1.8 centimorgans (cm) above RPP14, and pAt3-89.1, positioned 1.4 cm below RPP14, as described by Reignault et al. (1996). F2 plants that were either homozygous or heterozygous at RPP14 were then used for mapping the position of EDS1 relative to CAPS (Konieczny and Ausubel, 1993) and microsatellite (Bell and Ecker, 1994) markers, Linkage to GL1 and other markers on chromosome 3 was identified as shown in Table 2. No linkage was found to markers on chromosomes 1, 2, 4, and 5 (see Table 2). Examination of the recombination frequencies between EDS1 and chromosome 3 markers and evidence of recombination break points in individual F3 families placed the gene in a 25-cM interval below RPP14, 18 cm above BGL1 and 7 cm below m249.

eds1 Suppresses the Function of RPP14 and Several Other RPP Genes in Ws-0

Detailed phenotypic characterization of the response of Ws-eds1 seedlings to Noco2 in comparison to the wild-type resistant parent Ws-0 and the susceptible ecotype Col-5 was performed with seedlings that had been taken through one parental backcross. In contrast to the necrotic resistant response of Ws-0 cotyledons after inoculation with Noco2 (Figure 1A), cotyledons of 9-day-old Ws-eds1 seedlings supported early (visible 3 DAI) and heavy (by 7 DAI) asexual sporulation of Noco2 in the absence of visible plant cell necrosis (Figure 1B). Microscopic analysis of Ws-0 cotyledons stained with lactophenol trypan blue showed hypersensitive cell death of individual mesophyll cells penetrated by Noco2 24 hr after inoculation (Figure 1C). This contrasted greatly with Ws-eds1 cotyledons in which the Noco2 mycelium had grown unimpeded (Figure 1D). Three days after inoculation, further cell death had occurred in Ws-0 cotyledons, and no fungal development was observed beyond these necrotic areas (Figure 1E), whereas Ws-eds1 cotyledons were fully infected and the mycelium had grown extensively into the first true leaves (Figure 1F).

Previous genetic studies have identified several other RPP gene specificities in Ws-0. Each recognizes a particular isolate of P. parasitica, as shown in Table 3. Like RPP14, most of the specificities were mapped to chromosome 3. However, two mapped to chromosome 4. Therefore, it was worthwhile
Figure 1. Responses of Ws-0 and Ws-eds1 (EW80-1) to Inoculation with *P. parasitica* Isolate Noco2 and *P. syringae pv tomato*

(A) Incompatible reaction of a Ws-0 cotyledon 5 days after inoculation with a droplet of conidiospores from *P. parasitica* isolate Noco2. Necrotic lesions start to appear macroscopically after 3 days.

(B) Compatible reaction of a cotyledon of mutant Ws-eds1 5 days after inoculation with Noco2 conidiospores. Aerially borne conidiospores develop on sporangiophores growing out through the stomata. The first sporangiophores are detectable after 3 days.
related pathogens, we inoculatedWs-0 andWs-eds7seeds with isolates of several oomycete parasites that had been collected from other host species. These included P parasitica (downy mildew) from B. oleracea and Capsella bursa-pastoris, P tabacina (blue mold) from tobacco, Bremia lactucae (downy mildew) from lettuce, Albugo candida (white blister) from B. oleracea and Cardamine pratense, and A. tragopogonis (white blister) from Senecio vulgaris. All of these isolates were cultured on seedlings of their natural host species, and fresh conidiosporangia or zoospores (in the case of A. candida) were used to inoculateWs-0 andWs-eds7plants. Evidence of parasite reproduction was observed until 10 DAI. None of the parasite isolates sporulated on wild-typeWs-0, as shown in Table 4, although discrete lesions of flecking necrosis were typically formed as the result of attempted penetration by the parasite (data not shown).Ws-eds7did not support

to investigate whether theeds1mutation influenced RPP gene specificities in addition toRPP14by inoculatingWs-eds1with all of the availableWs-0incompatible isolates ofP parasitica. Cotyledons ofWs-eds1andWs-0seedlings were inoculated with these isolates and scored subsequently for asexual reproduction of the parasite.Ws-eds1was fully compatible, permitting heavy sporulation with each isolate. Thus, it is clear that theEDS1gene is essential for expression of downy mildew resistance conferred by severalRPPgenes inWs-0.

ed51Partially Suppresses Resistance toB. oleraceacompatible isolates ofP parasitica

All of theP parasiticaisolates described in Table 3 were derived originally from Arabidopsis. To test whether theeds1mutation had altered resistance to a wider range of closely

<table>
<thead>
<tr>
<th>Cross</th>
<th>F1 R S R S</th>
<th>χ² (1 d.f.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>EW80-1 xWs-0</td>
<td>21 0 97 34</td>
<td>(3:1) = 0.06</td>
</tr>
<tr>
<td>Col-5 xEW80-1</td>
<td>11 0 89 69</td>
<td>(9:7) = 1.26</td>
</tr>
</tbody>
</table>

* R, resistant; S, susceptible.

Chi-square values show that the observed resistance-to-susceptibility ratios do not deviate significantly from the indicated expected ratios (shown within parentheses) at P = 0.05 and 1 degree of freedom (1 d.f.).

Table 2. Frequency of Genetic Recombination betweenEDS1and Molecular Markers on Arabidopsis Chromosome 3

<table>
<thead>
<tr>
<th>Marker</th>
<th>Recombinant Chromosomes</th>
<th>Total Number of Chromosomes</th>
<th>Recombination Frequency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GapC</td>
<td>18</td>
<td>28</td>
<td>64</td>
</tr>
<tr>
<td>GAPA</td>
<td>15</td>
<td>32</td>
<td>47</td>
</tr>
<tr>
<td>GL1</td>
<td>20</td>
<td>56</td>
<td>37</td>
</tr>
<tr>
<td>pAT3-89.1</td>
<td>8</td>
<td>60</td>
<td>13</td>
</tr>
<tr>
<td>m249</td>
<td>4</td>
<td>60</td>
<td>7</td>
</tr>
<tr>
<td>BGL1</td>
<td>11</td>
<td>60</td>
<td>18</td>
</tr>
<tr>
<td>nga6</td>
<td>9</td>
<td>32</td>
<td>28</td>
</tr>
</tbody>
</table>

* Other CAPS or microsatellite markers tested showing no linkage toEDS1are NCC1, nga248, nga280, and nga111(chromosome 1); m246 andm429 (chromosome 2); SC5, 94539, andDHS1(chromosome 4); andASA1, nga76, nga129, andLFY3(chromosome 5).
reproduction by the parasite isolates from non-crucifer hosts, and the interaction phenotypes were indistinguishable from those of wild-type Ws-0.

Interestingly, all of the *P. parasitica* isolates derived from *B. oleracea* and *C. bursa-pastoris* were capable of low to moderate sporulation on Ws-eds1 (Table 4). We know that this sporulation was not caused by contaminant inoculum of isolates from Arabidopsis because spores were collected from the infected Ws-eds1 seedlings and used to inoculate seedlings of their original host. In each case, the non-Arabidopsis isolates of *P. parasitica* sporulated profusely on their natural host species. In addition, conidiosporangia of the *B. oleracea*-derived isolates are characteristically one and a half to two times greater in diameter than are the Arabidopsis-derived isolates (average diameter is ~12 µm). The larger spore size of the inoculum was observed throughout the experimentation with the *B. oleracea*-derived isolates. These isolates appear to be highly specialized for their respective host species, because 96 United Kingdom ecotypes of Arabidopsis were inoculated with each Brassicas-derived isolate and no sporulation was observed in any combination of host and parasite.

An isolate of *A. candida* from *B. oleracea* also sporulated asexually on Ws-eds1 seedlings by producing the characteristic white blisters. The blisters only formed on ~25% of the inoculated seedlings, and they appeared to be restricted in size (data not shown), suggesting that resistance to this isolate was also only partially suppressed by the eds1 mutation. This isolate was incapable of producing blisters in wild-type Ws-0.

**Ws-eds1 Exhibits Enhanced Susceptibility to *P. parasitica***

In several inoculation experiments, the amount of asexual sporulation by *P. parasitica*, visible as the characteristic downy

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**Table 3. Interaction Phenotype of Ws-eds1 after Inoculation with Different Homologous Isolates of *P. parasitica***

<table>
<thead>
<tr>
<th>Isolate</th>
<th>RPP Locusa</th>
<th>Chromosome (Interval)</th>
<th>Phenotypeb</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Ws-0</td>
</tr>
<tr>
<td>Emwa1</td>
<td>None</td>
<td>None (GL1 to m249)</td>
<td>EH</td>
</tr>
<tr>
<td>Noco2</td>
<td>14</td>
<td>3 (GL1 to m249)</td>
<td>PN</td>
</tr>
<tr>
<td>Cala2</td>
<td>10</td>
<td>3 (not mapped)</td>
<td>CN</td>
</tr>
<tr>
<td>Emoy2</td>
<td>1</td>
<td>3 (cosegregates with RPP14)</td>
<td>PN</td>
</tr>
<tr>
<td>Cand5</td>
<td>ND</td>
<td>3 (not mapped)</td>
<td>FN</td>
</tr>
<tr>
<td>Hiks1</td>
<td>(1)</td>
<td>3 (cosegregates with RPP14)</td>
<td>PN</td>
</tr>
<tr>
<td>Maks9</td>
<td>(1)</td>
<td>3 (cosegregates with RPP14)</td>
<td>PN</td>
</tr>
<tr>
<td>Wela3</td>
<td>12</td>
<td>4 (m580 to cer2)</td>
<td>FN</td>
</tr>
<tr>
<td>Ahco2</td>
<td>ND</td>
<td>4 (linked to RPP12)</td>
<td>FN</td>
</tr>
</tbody>
</table>

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**Table 4. Asexual Reproduction of Different Isolates of Plant Parasitic Oomycete Species on Ws-0 and Ws-eds1 Seedlings**

<table>
<thead>
<tr>
<th>Parasite Species</th>
<th>Original Host</th>
<th>No. Isolates Tested</th>
<th>Asexual Sporulationb</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>P. parasitica</em></td>
<td><em>B. oleracea</em></td>
<td>5</td>
<td>N</td>
</tr>
<tr>
<td></td>
<td>Capsella-bursa pastoris</td>
<td>1</td>
<td>N</td>
</tr>
<tr>
<td></td>
<td>Cardamine pratense</td>
<td>1</td>
<td>N</td>
</tr>
<tr>
<td><em>P. tabacina</em></td>
<td>Nicotiana spp</td>
<td>1</td>
<td>N</td>
</tr>
<tr>
<td><em>B. lactucae</em></td>
<td>Lactuca sativa</td>
<td>13</td>
<td>N</td>
</tr>
<tr>
<td><em>A. candida</em></td>
<td>Cardamine pratense</td>
<td>2</td>
<td>N</td>
</tr>
<tr>
<td><em>B. oleracea</em></td>
<td>Nicotiana spp</td>
<td>1</td>
<td>N</td>
</tr>
<tr>
<td><em>A. tragopogonis</em></td>
<td>S. vulgaris</td>
<td>1</td>
<td>N</td>
</tr>
</tbody>
</table>

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a N, none; L, low (<10 sporangiophores per cotyledon); M, medium (10 to 20 sporangiophores per cotyledon). A fully compatible interaction would have been designated H for heavy (>20 sporangiophores per cotyledon).

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Table 5. Conidiosporangia Production of *P. parasitica* Isolates in Wild-Type Ws-0 and Ws-eds1 (EW80-1) Seedlings at Different Developmental Stages

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Inoculated Tissue (Age)</th>
<th>Harvest (DAI)</th>
<th><em>P. parasitica</em> Isolate†</th>
<th>Conidiosporangia per Seedling‡</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Ws-0</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Mean SE</td>
</tr>
<tr>
<td>1</td>
<td>Cotyledons (7 days old)</td>
<td>7</td>
<td>Emco5</td>
<td>3,333 43</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Ahco2</td>
<td>0 —</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Emoy2</td>
<td>0 —</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>P-006</td>
<td>0 —</td>
</tr>
<tr>
<td>2</td>
<td>Cotyledons and juvenile leaves (8 days old)</td>
<td>3</td>
<td>Emwa1</td>
<td>195 138</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Noco2</td>
<td>0 —</td>
</tr>
<tr>
<td>3</td>
<td>Cotyledons and juvenile leaves (9 days old)</td>
<td>6</td>
<td>Emwa1</td>
<td>14,695 3,122</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Noco2</td>
<td>0 —</td>
</tr>
<tr>
<td>4</td>
<td>Cotyledons and juvenile leaves (14 days old)</td>
<td>10</td>
<td>Emco5</td>
<td>13,900 320</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Ahco2</td>
<td>0 —</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Emoy2</td>
<td>0 —</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>P-006</td>
<td>0 —</td>
</tr>
</tbody>
</table>

† Experiments 1 and 4 were conducted simultaneously at Horticultural Research International by using 7- (before emergence of the first juvenile leaves) and 14-day-old seedlings, respectively. Experiments 2 and 3 were conducted simultaneously at the Sainsbury Laboratory by using 9-day-old seedlings for inoculation. At this age, the first juvenile leaves were just beginning to emerge.

‡ Days after inoculation when conidiosporangia were harvested.

Isolates Emco5 and Emwa1 are compatible with wild-type Ws-0. Isolates Emoy2 and Noco2 are recognized by different RPP genes (RPP1 and RPP14, respectively) on chromosome 3 in Ws-0. Ahco2 is recognized by an unnamed RPP gene on chromosome 4 in Ws-0. All of these isolates were originally collected from Arabidopsis; isolate P-006 was collected from *E. oleracea*.

For experiments 2 and 3, the means were calculated from five samples that each contained conidiosporangia from 20 infected seedlings. For experiments 1 and 4, the means were calculated from six samples that each contained conidiosporangia from 10 infected seedlings. Isolate P-006 consistently produced low numbers of sporangiophores on Ws-eds1, but the number of conidiosporangia produced could not be quantified (NQ). Dashes indicate that values were not measurable. The method for quantifying conidiosporangia is described in Dangl et al. (1992a).
avirulence gene \textit{avrB} derived from the soybean pathogen \textit{P. s. pv glycinea} (Innes et al., 1993). Therefore, we inoculated leaves of \textit{Ws-eds1} and wild-type \textit{Ws-0} plants with the pathogenic strain DC3000 or strain DC3000 expressing \textit{avrB} to assess whether the mutant was compromised in race-specific resistance to this bacterial avirulence gene. Leaves of plants were infiltrated with low concentrations of bacteria (10^5 colony-forming units per mL) and monitored for disease symptom development and growth of the bacteria in the inoculated leaves. Severe symptoms developed over 5 days in \textit{Ws-0} and \textit{Ws-eds1} inoculated with DC3000. Interestingly, the progression of chlorosis and subsequent necrosis of the infected tissue was 24 hr more advanced in \textit{Ws-eds1} than in \textit{Ws-0}, as shown in Figures 1G and 1H, again suggesting an enhanced susceptibility (see above) of \textit{Ws-eds1} to the virulent strain DC3000. Also, the growth rate of DC3000 in \textit{Ws-eds1} was marginally greater than in \textit{Ws-0}, although by day 4 the number of bacteria extracted from infected leaves was not significantly different, as shown in Figure 2.

\textit{Ws-0} and \textit{Ws-eds1} plants were equally resistant to DC3000 expressing \textit{avrB}, as measured by the absence of disease symptoms (Figures 1G and 1H) and by a 100- to 1000-fold reduction in bacterial number in inoculated leaves over 4 days in both plant lines (Figure 2). Leaves were also inoculated with a concentration of bacteria (5 \times 10^7 colony-forming units per mL) that gave rise to a macroscopically visible, local hypersensitive plant reaction in the case of the avirulent strain DC3000 expressing \textit{avrB} on \textit{Ws-0} (Innes et al., 1993). This was clearly distinguishable at 16 to 20 hr from the virulent strain DC3000 on \textit{Ws-0}, which was symptomless at this early time point (results not shown). The timing and appearance of the HR were the same in \textit{Ws-0} and \textit{Ws-eds1} (data not shown). As a control, leaves of the natural \textit{avrB}-susceptible ecotype Blanes (Bl-2; Innes et al., 1993) were inoculated under the same conditions to test the pathogenic competence of DC3000 expressing \textit{avrB}, and these produced disease symptoms similar to \textit{Ws-0} (results not shown). Therefore, we concluded that \textit{Ws-eds1} retains full resistance to a \textit{P. syringae} avirulence gene \textit{avrB}, although it appears to allow more rapid disease lesion formation than does the wild-type plant with the virulent strain DC3000 of \textit{P. syringae}.

\textbf{Ws-eds1 Retains Responsiveness to the Chemical Inducer of Systemic Acquired Resistance, 2,6-Dichloroisonicotinic Acid}

An inducible plant defense mechanism known as systemic acquired resistance (SAR) has been analyzed in detail in tobacco and Arabidopsis plants (Ryals et al., 1994). It is activated by exposure of the plants to necrosis-inducing pathogens and leads to the accumulation of endogenous salicylic acid (SA), a vital component of SAR, increased expression of pathogenesis-related genes, and systemic resistance to a broad spectrum of pathogens (Ward et al., 1991; Gaffney et al., 1993; Uknes et al., 1993; Delaney et al., 1994). SAR is also fully induced by the exogenous application of the chemical compound 2,6-dichloroisonicotinic acid (INA; Uknes et al., 1992), which acts as an analog of SA or downstream of SA perception or binding (Vernooij et al., 1995). Therefore, we tested whether \textit{Ws-eds1} was compromised in its ability to activate SAR after INA application by measuring its response to the genetically compatible \textit{P. parasitica} isolate Emw1 and to the \textit{Ws-eds1}-compatible isolate Noc2. INA was sprayed onto seedlings 2 days before spray inoculating with Emw1, Noc2, or water. The results in Table 6 show that INA treatment induced almost complete resistance to Emw1 in both wild-type \textit{Ws-0} and \textit{Ws-eds1} leaves and to Noc2 in \textit{Ws-eds1} leaves. These tests indicated that the SAR response pathway, from the point of perception of the inducer INA, is intact in \textit{Ws-eds1}.

\textbf{DISCUSSION}

By using a mutational approach with Arabidopsis, we have identified a gene, \textit{EDS1}, that is essential for the expression of several naturally polymorphic \textit{RPP} genes required for race-specific resistance to the biotrophic parasite \textit{P. parasitica}. The \textit{RPP} genes influenced by the \textit{eds1} mutation are located on at least two Arabidopsis chromosomes and specify a range

\begin{figure}[h]
\centering
\includegraphics[width=0.5\textwidth]{figure2}
\caption{Growth of Virulent and Avirulent Strains of \textit{P. s. tomato} in Leaves of \textit{Ws-0} and \textit{Ws-eds1} (EW80-1). Leaves of \textit{Ws-0} (○ and △) or \textit{Ws-eds1} (□ and ▲) were infiltrated with 10^5 colony-forming units (cfu) per mL of the virulent strain DC3000 or the avirulent strain DC3000 expressing \textit{avrB}. Concentrations of viable bacteria were measured in the leaves at various time points after inoculation.}
\end{figure}
**Table 6. Development of P. parasitica Isolates Emwa1 and Noco2 in Ws-0 and Ws-edsl Seedlings Pretreated with INA**

<table>
<thead>
<tr>
<th>DA1 isolate</th>
<th>Asexual Sporulation&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Ws-0</th>
<th>Ws-edsl</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>- INA&lt;sup&gt;b&lt;/sup&gt;</td>
<td>+ INA</td>
<td>- INA</td>
</tr>
<tr>
<td>Emwa1</td>
<td>3</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Noco2</td>
<td>4</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Emwa1</td>
<td>7</td>
<td>+ +</td>
<td>(+)</td>
</tr>
<tr>
<td>Noco2</td>
<td>7</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

<sup>a</sup>The number of sporangiophores was assessed on individual leaves of at least 12 plants per treatment: -, none; (+), rare (1 to 5 on (5 to 10 on < 50% of the leaves); +, medium (5 to 20 on >75% of the leaves); ++, heavy (>25 on >90% of the leaves).

of interaction phenotypes in Ws-0 with respect to infections by corresponding isolates of P. parasitica. In light of recent advances in understanding the molecular basis of disease resistance, we propose that the EDS1 gene product is a necessary component in the signal transduction pathways that are specified by several but not necessarily all RPP genes.

Crosses have been initiated between Ws-edsl1 and selected ecotypes of Arabidopsis to determine whether other RPP genes, identified in these ecotypes, also require a functional EDS1 gene. Recently, a second edsl allele has been identified in the ecotype Ler-0. It exhibits complete suppression of Noco2 recognition conferred by the RPP5 gene located on chromosome 4 (J.E. Parker, unpublished data), reinforcing the notion that EDS1 encodes a common signaling component required by several different RPP genes. Interestingly, however, this mutant allele does not fully suppress the function of all RPP genes in Ler-0 (E.B. Holub and J.E. Parker, unpublished data). These results suggest that there may be RPP gene-mediated resistance pathways that are, at least in part, independent of EDS1 function. A more detailed examination of individual RPP gene-specified phenotypes in Ler-edsl1 and wild-type Ler-0 plants is now in progress. Also, transfer of RPP genes from the Ler-0 to the Ws-edsl background using marker-assisted selection will allow us to test more rigorously whether functional differences in specificity can occur between mutant alleles of EDS1.

The edsl mutation also causes a reduction in the level of resistance exhibited by Ws-0 to isolates of P. parasitica that are pathogens of B. oleracea. Therefore, we concluded that EDS1 may be required for so-called nonhost resistance to these particular P. parasitica isolates. This suggests that functionally common defense signaling components may exist between these two crucifer species in response to P. parasitica challenge. It follows that EDS1-responsive RPP genes are present in the Arabidopsis genome and that they recognize P. parasitica pathogens of B. oleracea. This promises well for intergeneric transfer of potentially useful disease R genes between Arabidopsis and Brassica species.

The intermediate phenotype of Ws-edsl1, after inoculations with B. oleracea-derived isolates, may reflect a deficiency in compatibility factors required for full pathogenesis of a heterologous P. parasitica isolate on Arabidopsis. More importantly, it suggests the presence of at least another gene in Ws-0 that is nonresponsive to EDS1 and required for resistance to heterologous isolates. A possible alternative explanation is that this particular edsl1 mutant allele intrinsically has a less severe effect on nonhost P. parasitica–triggered resistance. Several other oomycete parasite species, such as B. lactucae, which causes downy mildew on lettuce (Michelmore et al., 1988), were not able to cause infection in Ws-edsl1 (Table 4); it may be that these are evolutionarily less related to the Arabidopsis- and B. oleracea–infecting P. parasitica isolates tested here. The data, however, demonstrate a link between race-specific and nonhost disease resistance, as has been shown in earlier studies with several P. syringae avr genes (Kobayashi et al., 1989; Whalen et al., 1991; Dangl et al., 1992b; Fillingham et al., 1992; Innes et al., 1993; Wood et al., 1994).

Several recently characterized defense response mutations in Arabidopsis are instructive for anticipating the possible role of EDS1 in disease resistance. The first mutation, ndr1 (Century et al., 1995), suppresses resistance in Col-0 to four P. syringae avirulence genes, avrRpt2, avrB, avrRpm1, and avrPph3, as well as exhibiting variable suppression of resistance to several incompatible isolates of P. parasitica. This provides important evidence for the existence of a common step in resistance to prokaryotic and eukaryotic pathogens, and supports the hypothesis that convergent signaling pathways exist downstream of events presumably triggered by different R genes. Our studies clearly show functional differences between EDS1 and NDR1. Unlike Col-0 plants containing the ndr1 mutation, Ws-edsl1 seedlings retain full resistance to the bacterial avirulence gene _avrB_ from _P. syringae_. This is demonstrated by suppression of avirulent bacterial growth and the absence of disease symptoms at low inoculum concentrations that are comparable to wild-type Ws-0 plants. The avirulent bacterial strain was also capable of inciting a hypersensitive (HR) necrotic lesion at high inoculum doses in Ws-edsl1 with the same timing as in Ws-0. A fuller spectrum of bacterial avirulence genes, such as _avrRpm1_, _avrRpt2_, and _avrPph3_ (Century et al., 1995) as well as _avrRps4_ (Hinsch and Staskawicz, 1996), will be tested on both the Ws-edsl1 and Ler-edsl1 plants to assess the dependence of the corresponding _R_ genes on EDS1 function. Experiments have also been initiated to compare the requirements for NDR1 and EDS1 by the same spectrum of RPP genes in near isogenic backgrounds. This will enable us to assess more critically whether their signaling functions overlap or are mutually exclusive.

Two other recessive mutations, _npr1_ (for nonexpresser of pathogenesis-related genes; Cao et al., 1994) and _nim1_ (for
noninducible immunity; Delaney et al., 1995), identify gene products that are required for the SAR response in Arabidopsis and are postulated to be at the site of SA or INA activation of systemic defense responses or at some point downstream. Interestingly, Col-npr1 seedlings exhibit strong suppression of several RPP genes (E.B. Holub, unpublished data) as well as suppressing resistance to P. syringae (Cao et al., 1994). Delaney et al. (1995) also provide evidence for a requirement of the wild-type NIM1 gene in race-specific resistance. In their study, local, genetically determined resistance to Noco2, in the absence of SAR-inducing agents, is relaxed in nim1 when compared with Noco2 resistance in the wild-type Ws-O plant.

Our study shows that Ws-edsl plants are fully responsive to INA, becoming systemically resistant to the normally compatible P. parasitica isolate Emw1 and to the Ws-edsl-compatable isolate Noco2. Therefore, edsl identifies a signaling component that appears to be distinct from NIM1 or NPR1. The results are consistent with the SAR pathway being intact in Ws-edsl plants, although the phenotype does not rule out the possibility that edsl leads to a reduction in endogenous SA levels, which would be compensated for by the application of INA (Venooij et al., 1995). However, this is unlikely because it would result in an overall suppression of race-specific resistance to avirulent bacterial and fungal isolates, which has been demonstrated in plants engineered to degrade SA (Gaffney et al., 1993; Delaney et al., 1994). We show here that although the enhanced susceptibility phenotype of Ws-edsl to virulent isolates of P. parasitica is reminiscent of the response of SA-depleted Arabidopsis plants (Delaney et al., 1994), resistance is retained to the genetically incompatible P. s. tomato strain expressing avrB.

In summary, it race-specific resistance can occur in plants as a series of converging pathways that begin with specific recognition of the pathogen, which in some way activate defense responses, then it is likely that EDS1 controls a common step downstream of several RPP genes but upstream of more broadly convergent events identified by mutations, such as nim1 and npr1, or possibly ndr1, and acts at a step before SA accumulation and SA-activated responses. It will be instructive to measure both endogenous SA levels and the accumulation of pathogenesis-related protein transcripts in Ws-edsl plants under various inducing and pathogen-challenged conditions to test this model. Currently, this is certainly a far too simplistic model of biochemical events. Indeed, there is no proof that the R genes themselves are the primary recognition targets for corresponding pathogen Avr genes; therefore, potential signaling proteins such as EDS1 and NDR1 may operate upstream or in concert with R gene products. Additional genetic experiments are certainly required to determine the influence of pairwise mutations of genes such as EDS1, NDR1, NIM1, and NPR1 on a wide spectrum of R genes in Arabidopsis.

Mutagenesis in several plant–pathogen systems has been a powerful tool in identifying genes that are required for the function of race-specific R genes (Holub and Beynon, 1996; Kunkel, 1996; Schulze-Lefert et al., 1996). In barley, screening a mutagenized line containing the powdery mildew R gene Mla12 for susceptible mutants led, in addition to numerous defective Mla12 alleles, to the identification of two loci (Rar1 and Rar2) that are necessary components of Mla12-specified resistance (Freialdenhoven et al., 1994). In tomato, screening for mutations susceptible to Pto-mediated resistance to bacterial speck disease led to the discovery of a second tightly linked gene, Prf, that is required both for Pto gene function and for the function of a second linked gene, Fen, that controls sensitivity to the herbicide fenthon (Salmeron et al., 1994, 1996). In tomato, an elegant screen for increased fungal biomass was used in mutagenized plants to identify two loci, Rcr1 and Rcr2, that are necessary for Cf-9–specified resistance to the leaf-spotting fungus Cladosporium fulvum (Hammond-Kosack et al., 1994).

It is likely that different and more refined screens for mutants will reveal other genes involved in disease resistance signaling mechanisms, assuming there is not a great degree of redundancy. The activation of signal–response coupled reactions triggered by specific pathogen recognition also implicates the participation of negative regulatory circuits to prevent uncontrolled stimulation of the plant's defenses (Schulze-Lefert et al., 1996). The appropriate mutational screens have identified putative negative regulatory genes in Arabidopsis (Bowling et al., 1994; Dietrich et al., 1994; Greenberg et al., 1994) and in barley (Freialdenhoven et al., 1996).

To date, it is unclear to what extent there is cross-signaling between pathways leading to the arrest of development of an avirulent pathogen and mechanisms that limit growth of a virulent pathogen. SA-depleted tobacco and Arabidopsis plants (mentioned above) showed increased growth of both avirulent and virulent pathogens (Delaney et al., 1994), implicating SA as a common signal in these two responses as well as in SAR. Also, npr1 plants permitted greater ingress of a virulent P. syringae strain than did wild-type Col-0 (Cao et al., 1994), although it is unclear from that study whether any race-specific bacterial resistance was affected in the mutant plants. Several RPP genes, however, are influenced by npr1 (Holub and Beynon, 1996). Two Arabidopsis pad mutants, deficient in camalexin accumulation, allowed increased growth of virulent P. syringae strains but were resistant to avirulent strains (Glazebrook and Ausubel, 1994), suggesting that certain biochemical processes are unique to each response. In a recent study by Glazebrook et al. (1996), mutagenized Arabidopsis plants were screened for enhanced growth of a pathogenic P. syringae strain, and 10 different loci involved in limiting the spread of the disease lesion were found. Some of these loci were designated eds2 to eds8. Interestingly, defective alleles of Npr1 (Cao et al., 1994) and Pad2 (Glazebrook and Ausubel, 1994) were among the isolated mutants, supporting the idea that certain signaling components play a role in multiple defense pathways.

Our study shows that Ws-edsl allowed more rapid development of two Ws-O–compatible P. parasitica isolates than did wild-type Ws-0 plants (Table 5). However, this trait appears to be dependent on the age of the plants; very young seedlings
susceptibility. The apparent increased susceptibility during old growth stages of Ws-eds1 may be a consequence of greater surface area when inoculated and the larger biomass of host tissue for colonization. Ws-edsl also exhibited more rapid symptom expression of virulent P. s. tomato DC3000 (Figure 2), although this was correlated with only a marginal increase in the rate of bacterial growth (Figure 2). Also, the disease lesion did not extend farther than in wild-type leaves. It is possible that the accelerated symptom development observed in Ws-eds1 plants compared with wild-type Ws-0 under these experimental conditions is disproportionately greater than the limited enhancement of bacterial growth.

Altogether, our results suggest that the wild-type EDS1 gene has some influence in restricting development of certain virulent pathogens as well as playing a crucial role in RPP gene-mediated resistance, drawing another possible link between pathways limiting pathogen development in both genetically incompatible and compatible interactions. Near-isogenic lines will be invaluable in making further comparisons and in ruling out possible effects of genetic background in the host and/or parasite. Experiments are in progress to test whether Ws-eds1 is allelic with any of the other eds mutations identified by Glazebrook et al. (1996).

EDS1 is a suitable target for molecular isolation either by positional cloning or by transposon tagging. Preliminary mapping of EDS1 shows that it is linked to the cluster of RPP specificities residing on chromosome 3 between Gl1 and m249 (13% recombination with pAT3-89.1 [Table 2], a marker that was positioned 1.4 cm from RPP14 in a previous study [Reignault et al., 1996]). However, linkage is not tight in contrast to the relative positions of the tomato Prf and Pto genes (Salmeron et al., 1996). Cloning the EDS1 gene as well as performing further genetic analysis of Ws-eds1 in combination with different R genes and resistance signaling mutants in Arabidopsis should allow us to establish its biochemical role in plant–pathogen interactions.

METHODS

Maintenance of Bacterial Strains and Fungal Isolates

Pseudomonas syringae pv tomato DC3000 containing the avirulence gene avrB in the broad host range vector pVSP61 (Innes et al., 1993) and containing pVSP61 without an insert was kindly provided by B. Staskawicz (University of California at Berkeley). These were cultured as described previously (Innes et al., 1993). Isolates of Peronospora spp, Albugo spp, and Bremia lactucae were cultured on their corresponding compatible host ecotypes, as described previously (Dangl et al., 1992a; Holub et al., 1994, 1995). The other host species were grown in the same manner as Arabidopsis thaliana, and all parasite inocula were prepared and applied as was done for similar isolates from Arabidopsis. B. lactucae was maintained on lettuce cultivar Cobham Green; Brassica-derived P. parasitica was maintained on B. oleracea cv Maris Kestral; P. tabacina was maintained on Nicotiana rustica; and the other isolates were maintained on wild-type, uncharacterized ecotypes.

The P. parasitica isolates from Arabidopsis were described previously (Parker et al., 1993; Holub et al., 1994; Holub and Beynon, 1996). All were derived from single oospores (sexual stage), except for Noco2, which was maintained as a mass conidiosporangia culture derived originally from a single host seedling. Two isolates of P. parasitica from B. oleracea (P-005 and P-006) were described by Moss et al. (1994). The isolate of A. candida from B. oleracea (Ac9) was obtained from the Crucifer Genetics Cooperative (University of Wisconsin, Madison). Isolates of B. lactucae were provided from the collection of I.R. Crute (Horticultural Research International) and included B5/92, B7a/84, B12/88, B28/94, B30/94, B32/94, IL4, S1, SF1, SF5, VD11, T, and B7a/84. The remaining isolates were uncharacterized mass spore cultures collected by E.B. Holub from single leaves of host plants found in the United Kingdom.

Plant Material, Cultivation, and Pathogenicity Tests

Seed of the Arabidopsis ecotype Wassilewskija (Ws-0) were originally obtained from K. Feldman (University of Arizona, Tucson). M2 seed stocks generated from ethyl methanesulfonate–mutagenized Ws-0 seed were a kind gift of F. Chumley (Du Pont, Wilmington, DE). Seed of ecotype Columbia (Col-5, containing the recessive mutation gff) was obtained from J. Dangl (University of North Carolina, Chapel Hill). Seed of the ecotype Blanes (Bl-2) were obtained from the Nottingham Arabidopsis Stock Centre (Nottingham, UK). The collection of 96 United Kingdom ecotypes used for testing the pathogenicity of Brassica-derived P. parasitica isolates was assembled by E.B. Holub from wild populations throughout Britain.

Ethyl methanesulfonate–mutagenized M2 seedlings were sown at a density of ~400 seed per 320 cm². These seedlings and Col-5 × Ws-eds1 F2 families (25 to 30 seedlings) were inoculated by spraying a suspension of 4 × 10⁴ mL of P. parasitica conidia on run off. Inoculated seedlings were incubated at 16°C. Susceptible plants were rescued as previously described (Reignault et al., 1996). Dichloroisocianic acid (DIA), obtained as a 25% active ingredient wettable powder formulation, was a gift from E. Ward (Ciba-Geigy Corp., Research Triangle Park, NC). INA or the control carrier powder formulation was sprayed onto seedlings at a concentration of 25 mg/L to imminent runoff. Seedlings were then spray inoculated with P. parasitica spores 2 days after INA treatment, as described above. Conditions for the growth of plants and bacteria for bacterial inoculations, infiltration of leaves with bacterial suspensions, and in-the-plant bacterial growth assays were as described by May et al. (1996).

Light Microscopy

Development of P. parasitica Noco2 was observed in whole infected cotyledons and leaves. Staining was with lactophenol trypan blue followed by destaining with chloral hydrate, as described previously (Koch and Slusarenko, 1990). The lactophenol trypan blue stain is selectively retained by fungal tissue as well as by dead plant cells (including xylem vessels of the vascular tissue) or cells that have sustained membrane damage (Keogh et al., 1990). Material was mounted in chloral hydrate and examined using phase-contrast optics on a Zeiss Axioskop microscope (Carl Zeiss Ltd., Welwyn Garden City, UK).
Isolation of Plant Genomic DNA, Molecular Markers, and Mapping Analysis

Plant genomic DNA was isolated from flowering plants using the rapid flower head preparation method of Martienssen et al. (1989), as described by Reignault et al. (1996). DNA prepared in this way was suitable for restriction enzyme digestion and polymerase chain reaction analysis. Polymerase chain reaction analysis of genomic DNA sequences was performed according to Reignault et al. (1996). Oligonucleotide primers were as described by Konieczny and Ausubel (1993) to generate codominant cleaved amplified polymorphic DNA sequence (CAPS) markers. Primers to amplify microsatellite sequences were designed according to Bell and Ecker (1994). Primer sequences to amplify a portion of m249 were kindly provided by P. Bittner-Eddy and J. Beynon (Wye College, Ashford, UK). The amplification products in Ws-O (1.9 kb) and Col-5 (2.5 kb) exhibited a simple sequence length polymorphism. The restriction fragment length polymorphism marker pAT349.1 (Shirley et al., 1992) was obtained from B. Shirley (Virginia Polytechnic Institute and State University, Blacksburg, VA). Restriction digests, DNA gel blotting, phosphorus-32 labeling, and hybridizations were performed according to standard protocols (Ausubel et al., 1987). In the mapping analysis, the percentage of recombinations between markers in selected Col-5 × Ws-ed3 F2 plants or corresponding F3 families was examined to identify linkage groups.

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