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Received 18 July 1995. Accepted 10 October 1995.

*Plasmodiophora brassicae*, the causal agent of clubroot, is known to be pathogenic on *Arabidopsis thaliana*. We identified for the first time pathotype-specific resistance to clubroot in *Arabidopsis*. Ecotypes Ts-0 and Ze-0 showed incompatible interactions to one isolate (isolate e) of the pathogen, but not three others tested. The resistance reaction is characterized by the lack of typical clubroot swellings after infection. Microscopical investigations revealed that resistance is accompanied by a hypersensitive reaction (HR). Cell wall alterations associated with the HR were detectable as autofluorescent substances. Positive phloroglucinol-HCl staining suggests a lignification of cell walls involved in defense reaction. By genetic analysis, we found that resistance to *P. brassicae* isolate e is conferred by a dominant allele of a single nuclear gene that we designated *RPBI*. With the aid of morphological markers this locus was mapped near the marker dis2 to chromosome 1.

Additional keyword: necrosis.

*Plasmodiophora brassicae* Woronin is a soilborne, obligate plant pathogen that causes the clubroot disease of crucifers. The life cycle of the parasite consists of two main phases, the first occurring in root hairs, and the second in cells of the cortex and stele of the root. During the latter phase, multinucleate plasmodia stimulate the invaded host cells and adjacent cells to grow and divide, leading to the development of the characteristic galls. Mature plasmodia produce resting spores that after rotting of the galls are released into the soil where they remain viable for several years. *Plasmodiophora brassicae* causes serious losses in *Brassica* crops and is difficult to control. Resistance breeding as a measure to control clubroot is complicated by the occurrence of many different pathotypes of the parasite. Pathotype-specific host resistances are found in different *Brassica* genotypes and the genetics of resistance is known for some of them. Resistance in *Brassica campestris* and *Brassica napus* is controlled by monogenic dominant systems, whereas resistance in *Brassica oleracea* is mainly recessive and governed by several genes (Crute et al. 1980).

Very little is known about the mechanisms that make plants resistant to *P. brassicae*. Although early speculations exist on morphological, anatomical, or biochemical characteristics of plants, which could influence the disease reaction to *P. brassicae* (reviewed by Karling 1968), none of them could provide a general resistance mechanism to clubroot. Butcher et al. (1974) showed that susceptibility of certain *B. campestris* plants appeared to be related to the presence of the glucosinolate glucobrassicin acting as an auxin precursor. But the proposal that the absence of glucobrassicin reduces symptoms of clubroot could not be generalized for other hosts (Ockendon and Buczacki 1979; Mullin et al. 1980). In conclusion, though clubroot resistance may be influenced in some cases by one or more of these factors, there is still no explanation of the mechanism by which some plants can resist infection by certain pathotypes of *P. brassicae* but show severe symptoms when infected by others.

In many plant-pathogen interactions, the resistant host develops defense mechanisms resulting in a hypersensitive reaction (HR) (Müller 1959; Klement 1982). The HR is characterized by the death of a few cells surrounding the site of pathogen penetration, leading to the inhibition of further spread of the pathogen. HR involves a series of dramatic changes in cell physiology (Dixon and Lamb 1990) that include an accumulation of phenolic compounds (Matern and Kneusel 1988) and alterations in cell wall metabolism (Vance et al. 1980). In the literature on *P. brassicae* only one report provides evidence for the occurrence of an HR associated with clubroot resistance of a *B. campestris* genotype (Dekhuijzen 1979).

Because of the lack of identified products of resistance genes, the only way to gain insight into their possible function implies their isolation and the prediction of the putative gene products from the DNA sequences. This has been recently achieved for several resistance genes, including the maize gene *Hm1* (Johal and Briggs 1992), which confers resistance to the fungus *Cochliobolus carbonum*, the tomato *Cf-9* gene for resistance to *Cladosporium fulvum* (Jones et al. 1994), and genes that specify resistance to the bacterial pathogen *Pseudomonas syringae* in tomato (Martín et al. 1993) and in *Arabidopsis* (Bent et al. 1994; Mindrinos et al. 1994). The advantages of *Arabidopsis* with respect to crop plants for cloning genes have been repeatedly emphasized.

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(e.g., Meyerowitz 1989), and led to the search for pathogens of this species and respective resistances. Therefore, this plant has been established as a model host for several bacterial (Simpson and Johnson 1990; Davis et al. 1991; Debener et al. 1991; Whalen et al. 1991), viral (Melcher 1989), fungal (Koch and Slausarenko 1990; 1990b), and nematode (Stijmons et al. 1991) plant pathogens and is now widely used for investigations in pathogen interactions in order to explore the molecular basis of plant defense.

Colioun (1958) listed Arabidopsis as a host of P. brassicae, based on a report by Naumov (1925). More recent reports confirmed the susceptibility, but utilization of Arabidopsis as a tool for molecular studies of resistance to clubroot has been hindered so far because no clear variation in disease reaction was found (Koch et al. 1991; Mithen and Magrath 1992). This study was conducted with the objective of finding natural variation among different Arabidopsis ecotypes in their resistance response to P. brassicae isolates and characterizing the type of resistance response. Genetic analysis demonstrated the monogenic inheritance of the resistance reaction. Furthermore, as a first step to closely map the resistance gene relative to molecular markers, a chromosomal localization of the gene of interest has been done with the aid of morphological markers.

RESULTS

Natural variation in resistance to clubroot.

Thirty Arabidopsis ecotypes of diverse geographical origin (for a listing of the ecotypes tested, see Materials and Methods) were screened for their interaction with four P. brassicae isolates differing in their pathogenicity on cultivated Brassicas (isolates a, b, e, and k, see Table 1). Resistance tests initially were performed in the greenhouse, where 15 to 19 plants of each ecotype were inoculated with each isolate. All P. brassicae isolates tested were generally able to induce clubroot symptoms on Arabidopsis. However, symptom development was not always uniform for all ecotypes, i.e., apart from clearly susceptible individuals, plants without symptoms were also observed in some ecotypes. Resistance tests of the progeny from individual plants without or with weak symptoms, however, did not verify a decreased susceptibility and it is assumed that the plants from which the tested progeny were derived had escaped infection. Therefore, ecotypes that were not uniformly symptomless have been considered to be susceptible, because the heterogeneity was not due to genetic differences but was rather influenced by environmental factors. As a consequence, all resistance tests after this initial screening were performed in growth chambers under controlled conditions, which resulted in more homogeneous reactions.

In our studies, no resistance reaction at all was found in any ecotype after inoculation with isolates a, b, and k. In contrast, stable, pathotype-specific resistances were found to isolate e that were observed in the greenhouse as well as in the growth chambers. Ecotypes Tsu-0 and Ze-0 showed no clubroot swellings after inoculation with this isolate, in contrast to 28 other ecotypes tested, all of which developed the typical disease symptoms. Representative interactions of two Arabidopsis ecotypes (Cvi-0 and Tsu-0) with two P. brassicae isolates (isolate e, isolate k) are shown in Figure 1.

Phenotypic characterization of the compatible interaction.

Most interactions between A. thaliana ecotypes and P. brassicae isolates were defined as compatible. In these ecotypes, first visible disease symptoms became apparent as a swelling of the hypocotyl 2 weeks after inoculation. Ten to 14 days later, when plants were dug out for examination, susceptible ecotypes showed typical clubroot symptoms, i.e., severe swelling of hypocotyl, main root, and lateral roots (Fig. 1 A,B,E). Infected plants often were stunted and delayed in flowering, and some died before flowering. Microscopical investigations revealed that infection promoted massive cell divisions and cell enlargements resulting in a disorganization of tissue structure, as described by Mithen and Magrath (1992). The fungus colonized the whole root and started sporulation 2 to 3 weeks after inoculation. In some ecotypes, symptoms of P. brassicae infection were also observed on shoots (swelling, deformation, and stunting of shoots), a phenomenon that was more frequent when plants were challenged with a higher spore load. Light microscopy revealed the presence of P. brassicae resting spores in all infested tissues.

Phenotypic characterization of the incompatible interaction.

Ecotypes Tsu-0 and Ze-0 showed clear incompatible interactions with isolate e. Their resistance reaction is characterized by the absence of typical clubroot symptoms (abnormal swellings of root and hypocotyl), a slight reduction in number of lateral roots, and the occurrence of host cell necrosis. Necrotic tissues are macroscopically visible as brown spots on root surfaces, as shown in Figure 2.

Light microscopical investigations of the main root revealed that, in contrast to susceptible ecotypes, the general root anatomy remained unchanged, without the occurrence of abnormal cell divisions. Furthermore, groups of root cortical cells could be distinguished from adjacent translucent cells by their brownish color (Fig. 3A). Such groups of brownish cells were seen in neither susceptible nor noninoculated plants. These necrotic cells displayed the cytological features of an HR, which resulted in the restriction of fungal growth within the necrotic areas. Fluorescence microscopy of unstained root sections gave further information about the events at infection sites. At wavelengths of 420 to 490 nm, the cell content of invaded cells appeared orange in color and a strong autofluorescence of cell walls was detected, suggesting chemical changes in cell wall composition (Fig. 3B). Fluorescent cell walls appeared thickened. Autofluorescence was observed in cell walls of necrotic cells, but was strongest in adjacent living cells in close contact with the necrotic cells. A weaker

| Table 1. Pathogenic capabilities of Plasmoplasma brassicae isolates |
|-----------------------------|-----------------------------|
| Isolate | Isolate source | Attacks most of the hosts in group* | Less virulent against hosts in group* |
| a | Rapeseed | Brassica oleracea, B. napus | B. campestris |
| b | Rapeseed | B. oleracea | B. napus |
| e | Stubble turnip | B. campestris, B. napus | B. oleracea |
| k | Host 07* | B. oleracea, B. napus | B. campestris |

* European Clubroot Differential (ECD) set.
autofluorescence was observed, irrespective of infection, in cell walls of the outer layer of the periderm and in the central cylinder of the root (Fig. 3B). To learn more about the nature of the cell wall-associated substances, sections were stained with phloroglucinol-HCl. Viewed under bright field these sections showed that autofluorescent cell walls stained red, indicating the accumulation of lignin.

Weak swellings were occasionally observed in lateral roots of resistant ecotypes. Microscopical observations of these swellings showed the presence of the parasite in few cells, host cell enlargements, and cell divisions. However, development of the fungus was strongly inhibited and, in contrast to susceptible reactions, in which the whole root was colonized by the parasite, infected tissues were restricted to a very small area of the root system. Though the resistance mechanism proved to be incomplete in these parts of the root, the overall disease reaction was clearly distinguishable from that of the susceptible reaction, in which the fungus developed unimpeded and severe symptoms developed.

Both Tsu-0 and Ze-0 were resistant to isolate e but differed in their reaction phenotype. In Tsu-0 necrotic regions were more frequent and larger than in Ze-0. The occurrence of small nodules and tiny swellings, indicating limited development of the parasite, was more often observed in Tsu-0 than in Ze-0.

Genetics of the resistance to isolate e.

To determine the genetic basis of the differential response to isolate e we performed crosses of the resistant ecotypes Tsu-0 and Ze-0 to the susceptible ecotype Cvi-0. Ecotype Cvi-0 was chosen because of its very distinct clubroot symptoms. F1 plants were scored macroscopically for clubroot symptoms following inoculation with isolate e. All F1 plants were found to be resistant, as illustrated in Table 2. No mater-

![Fig. 1. Symptoms induced by Plasmodiophora brassicae isolates on Arabidopsis thaliana ecotypes. Reactions of ecotypes Cvi-0 and Tsu-0 are shown 25 days after inoculation with P. brassicae isolates e and k, demonstrating compatible and incompatible interactions. Clubroot symptoms are visible in the interaction of Cvi-0 with both isolates (A, B), whereas Tsu-0 only exhibits clubroot symptoms in the interaction with isolate k (E) but not with isolate e (D). Control plants (C, F) were not inoculated. Bar in A = 5 mm.](image-url)
nal effect was detected in reciprocal crosses. F₁ plants were allowed to self and the F₂ progeny was scored for its response to inoculation with isolate e. Resistance segregated in a 3(resistant):1(susceptible) ratio (Table 2). These results demonstrate that resistance to isolate e in ecotypes Tsu-0 and Ze-0 is determined by a dominant allele of a single gene. In a test for allelism, Tsu-0 and Ze-0 were crossed and the resulting F₂ generation was tested for resistance to isolate e. All 185 F₂ plants showed the incompatible reaction indicating that resistance in Tsu-0 and Ze-0 is governed by the same locus that we designated RPBI.

Mapping of the resistance gene RPBI in Tsu-0.

In order to map RPBI with the aid of morphological markers, crosses were made between Tsu-0 (resistant to isolate e) and four different marker lines (M4, M10, M11, and M13, all susceptible to isolate e) each carrying one or two recessive morphological markers. F₁- and F₂-progenies were scored for their disease reaction to isolate e. Although differentiation between resistant and susceptible reactions was not as clear as in the cross with ecotype Cvi-0, the monogenic, dominant inheritance of the resistance trait could be confirmed and segregation of the resistance trait and the marker genes hy2 (M4), ap2 (M10), tig (M11), an, and dis1 (both M13) could be determined. As seen in Table 3, in three of the four F₂ progenies segregation of marker genes and resistance genes was independent. In the F₂ progeny of marker line M13 a weak linkage to marker an and a stronger linkage to dis1 was observed. For dis1 and RPBI a recombination fraction of 0.21 was calculated. According to these data RPBI maps on the top of chromosome 1 and is located on the “classical” linkage map (Koornneef et al. 1983) in the vicinity of phenotypic marker dis2.

DISCUSSION

The most important prerequisite for the use of Arabidopsis as a model for the genetic analysis of plant pathogen interaction is the presence of compatible and incompatible reactions that should be as clear-cut as possible. In this study we observed well-defined disease symptoms as well as a clear resistance phenotype for the Arabidopsis/P. brassicae interaction. Until now, investigations on this interaction gave only slight differences in the degree of symptom expression (Koch et al. 1991; Mithen and Magrath 1992). Those investigations demonstrated that late flowering plants developed more severe symptoms than earlier flowering plants. Differences in disease reaction were attributed to the two competing carbon sinks of flower and gall. The phenotypic differences in disease reaction observed in the present study, however, were not dependent upon the time to flowering. Although ecotype Ze-0, for example, is late flowering (over 80 days until flowering) it shows resistance to isolate e, whereas the early flowering ecotype Cvi-0 (25 days until flowering) shows severe symptoms after inoculation with this isolate.

The high degree of incompatibility of Tsu-0 and Ze-0 with isolate e was manifested as necrosis of cortical cells reacting hypersensitively to the pathogen. Except for a report by Dekhuijzen (1979) on the occurrence of a hypersensitive host reaction in a resistant B. campestris genotype, there is no evidence that clubroot resistance is associated with an HR. Kroll et al. (1983) observed in clubroot-resistant radish plants that further growth of P. brassicae became arrested without the occurrence of an HR. Therefore, it remains unclear whether an HR is a common feature of clubroot resistance.

Table 2. Segregation of resistance to isolate e

<table>
<thead>
<tr>
<th>Cross</th>
<th>Generation</th>
<th>Resistant</th>
<th>Susceptible</th>
<th>Total</th>
<th>( \chi^2 )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tsu-0(R) × Cvi-0(S)</td>
<td>F₁</td>
<td>10</td>
<td>0</td>
<td>10</td>
<td>0.39 NS*</td>
</tr>
<tr>
<td></td>
<td>F₂</td>
<td>90</td>
<td>34</td>
<td>124</td>
<td></td>
</tr>
<tr>
<td>Ze-0(R) × Cvi-0(S)</td>
<td>F₁</td>
<td>15</td>
<td>0</td>
<td>15</td>
<td>0.07 NS*</td>
</tr>
<tr>
<td></td>
<td>F₂</td>
<td>86</td>
<td>27</td>
<td>113</td>
<td></td>
</tr>
</tbody>
</table>

* \( \chi^2 \) values calculated for expected ratio 3 resistant:1 susceptible.

* Not significant at \( P = 0.05 \).
We have demonstrated that resistance in ecotypes Tsu-0 and Ze-0 is associated with the accumulation of autofluorescent substances in cell walls of cells involved in the defense reaction. This phenomenon is known to also occur in other incompatible plant-pathogen interactions (Graham and Graham 1991) and probably indicates the biosynthesis of cell wall–associated phenolic compounds that are commonly produced during the defense response of the plant (Matern and Kneusel 1988; Nicholson and Hammerschmidt 1992). The positive Wiesner test (phloroglucinol-HCl stain) strongly suggests that the changes in cell wall composition are at least partially due to the formation of lignin. Lignification resulting in cell wall strengthening is thought to be a common response to pathogen attack (Vance et al. 1980).

Resistance of ecotypes Tsu-0 and Ze-0 is pathotype specific. Both ecotypes express resistance only to isolate e but exhibit severe clubroot symptoms when inoculated with isolates a, b, and k. A gradient of pathogenicity of P. brassicae isolates resulting in interactions ranging continuously from resistance to susceptibility, as observed for interactions with B. oleracea, was not observed in interactions with Arabidopsis ecotypes. Therefore, the interaction type between P. brassicae and Arabidopsis resembles the type observed in B. campestris and B. napus rather than that observed in B. oleracea, and this also applies to the genetics of the resistance. Resistance in B. napus and B. campestris is monogenic, whereas resistance in B. oleracea is polygenic (Crute et al. 1980).

Based on genetic analyses, we have identified the locus RPB1 in Arabidopsis that controls the resistance reaction to isolate e. The pathotype specificity of the resistance reaction, its monogenic inheritance, and the expression as an HR seem to indicate that resistance in the Arabidopsis/P. brassicae system is governed by a gene-for-gene interaction.

In most of the pathosystems using Arabidopsis as a model host, the parasite partner is amenable to molecular analysis. In our model system the investigation of the parasite part of the interaction causes difficulties. Plasmopodiphora brassicae is an obligate biotroph and cannot be cultivated on artificial media. Little is known about the sexuality of the fungus and, at present, it is not possible to cross isolates of different pathogenic capacities. Nevertheless, it is valuable to investigate the interaction of P. brassicae with Arabidopsis because insights provided by other model systems will not explain every host parasite association. Every addition of new Arabidopsis pathosystems showing variation in the host response and comparative studies on a wide variety of pathosystems will elucidate common principles as well as the differences in plant defense. Because of the importance of P. brassicae as a pathogen of cultivated Brassicas an attempt to isolate clubroot resistance genes from Arabidopsis could be of practical value for the control of the disease.

In conclusion, we have demonstrated for the first time the presence of a clear clubroot resistance phenotype in Arabidopsis that is accompanied by an HR. The high degree of incompatibility between host and parasite, the identification of a single locus conferring resistance, and the knowledge of the chromosomal position of the resistance gene are ideal prerequisites for a map-based approach to isolate the gene in question. We are currently mapping RPB1 relative to molecular markers with the objective of cloning this gene by chromosome walking. The anticipated isolation of RPB1 should contribute to the understanding of the function of clubroot resistance genes and the mechanisms by which plants can resist P. brassicae.

MATERIALS AND METHODS

Plant material and growth conditions.

The following ecotypes and mutant lines were obtained from the Arabidopsis Information Service Seed Bank in Frankfurt/M.; Germany (Kranz and Kirchheim 1987):

Ecotypes: Al-0, An-1, Bur-0, Bus-1, Can-0, Co-1, Col-0, Cvi-0, Ei-2, En-2, Est-0, Gr-1, Hi-0, Ito-0, Kas-1, La-0, Lm-2, Lu-1, Mt-0, Oy-0, Pa-1, Per-1, Sol-0, Sue-0, Sy-0, Tc-0, Tsu-0, Wil-1, Yo-0, and Ze-0. Mutant lines: M4, M10, M11, and M13.

To obtain uniform germination, seeds were moistened and stored for 4 days at 4°C. Cold treated seeds were placed on the surface of the substrate with the aid of forceps at a distance of approximately 3 cm between seedlings. The substrate, consisting of a 3:1 potting soil/sand mixture, was sieved through a 5-mm mesh and thoroughly moistened. Until germination, plant containers were covered with a clear plastic foil to maintain sufficient soil moisture. For the duration of the experiment the soil was moistened by placing plant containers on wetted fleece. For the initial resistance screening, ecotypes were grown in the greenhouse in clay pots (15 cm in diameter) at a density of 15 to 19 seedlings of each ecotype per pot. For all other experiments plants were grown at same densities (140 plants/box) in flat plastic containers (30 × 47 × 6 cm). Containers were placed in growth cabinets under a 16-h photoperiod at 21°C.

Table 3. Linkage analysis of RPB1 in F2 progenies of crosses between Tsu-0 and four marker lines

<table>
<thead>
<tr>
<th>Cross with marker line</th>
<th>Marker</th>
<th>RPB1/M.</th>
<th>RPB1/mm</th>
<th>rpb1 rpb1/M.</th>
<th>rpb1 rpb1/mm</th>
<th>Total</th>
<th>χ²</th>
<th>χ² &lt;sup&gt;abh&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>M4</td>
<td>hy2</td>
<td>63</td>
<td>20</td>
<td>13</td>
<td>9</td>
<td>105</td>
<td>2.46 NS&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>M10</td>
<td>ap2</td>
<td>61</td>
<td>22</td>
<td>19</td>
<td>6</td>
<td>108</td>
<td>0.06 NS&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>M11</td>
<td>tgt</td>
<td>50</td>
<td>18</td>
<td>15</td>
<td>7</td>
<td>90</td>
<td>0.24 NS&lt;sup&gt;c&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>M13</td>
<td>dis1</td>
<td>66</td>
<td>12</td>
<td>15</td>
<td>8</td>
<td>101</td>
<td>4.21 S&lt;sup&gt;d&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>70</td>
<td>8</td>
<td>10</td>
<td>13</td>
<td>101</td>
<td>23.09 S&lt;sup&gt;d&lt;/sup&gt;</td>
<td></td>
</tr>
</tbody>
</table>

<sup>a</sup> RPB1/M.: resistant plants not showing the morphological marker; RPB1/mm: resistant plants showing the morphological marker; rpb1 rpb1/M.: susceptible plants not showing the morphological marker; rpb1 rpb1/mm: susceptible plants showing the morphological marker.

<sup>b</sup> χ² values calculated for independent segregation of the resistance gene and the morphological marker.

<sup>c</sup> NS: Not significant at P = 0.05.

<sup>d</sup> Significant at P = 0.05.
Plasmodiophora brassicae isolates and preparation of inoculum.

All *P. brassicae* isolates originate from German Brassica growths and have different pathogenic capabilities on the European Clubroot Differential set (ECD-Set, Buczacki et al. 1975) as shown in Table 1 (after Diederichsen et al., in press). Isolates were provided by H. Busch (DSV, Thüle, Germany), M. Schoeller (Institut für Angewandte Genetik, Universität Hannover, Germany), P. Mattusch (Biologische Bundesanstalt für Pflanzenschutz im Gemüsebau, Hürth-Fischenich, Germany), and E. Diederichsen (Institut für Angewandte Genetik, Berlin, Germany).

Inoculum was prepared from mature clubs of Chinese cabbage. Clubroot galls were stored at −20°C until required. Resting spores were extracted by homogenizing clubroot galls, followed by filtering through gauze (25 μm pore width) and repeated centrifugation. The spore suspension was diluted to a concentration of 1 × 10⁶ spores/ml using a hemacytometer. As spores from different inoculum multiplications can differ in their aggressiveness, material from the same propagation was used for all experiments.

Inoculation of plants.

Twelve to 16 days after germination plants were inoculated by injecting 2 ml of a resting spore suspension into the soil in the root region of seedlings. For the first 10 days after inoculation the substrate was kept very wet to ensure optimal infection conditions. Disease reactions were scored 24 to 28 days after inoculation.

Microscopy.

Development of the fungus and resistance reactions were studied 3 to 4 weeks after inoculation in unstained and unfixed specimens. Cross sections and longitudinal sections of roots were made with the aid of a microtome (Vibratome 1000, Poly-Sciences Ltd., Eppelheim, Germany) after enclosing the roots in kneadable paraffin. Sections were viewed live and unstained on a Leitz Orthoplan microscope under bright-field or epifluorescence optics. Autofluorescing substances were identified using violet/blue excitation (excitation filter, 420 to 490 nm; dichroic mirror, 510 nm; and barrier filter, 515 nm).

Genetic analysis.

Emasculation of the female parent plant was performed under a dissection microscope on closed buds with the aid of forceps. Two to 3 days after emasculation donor pollen from the male parent plant was transferred to the mature stigma.

Reciprocal crosses between the susceptible ecotype Cvi-0 and the resistant ecotypes Tsu-0 and Ze-0, respectively, and segregation analysis of *F₂* progenies were carried out to determine the genetic basis for the resistance reaction.

Linkage analysis was performed utilizing progenies from crosses between ecotype Tsu-0 (resistant to isolate e) and marker lines each carrying recessive marker genes for morphological traits (hy2 in M4, ap2 in M10, t55 in M11, an and disl in M13) with well-established positions on the linkage map (Koornneef et al. 1983; Hauge et al. 1993). All marker lines were susceptible to isolate e. The homozygotes (*F₁*) and their selfed progenies (*F₂*) were inoculated with isolate e and scored for disease reaction and mutant phenotype. Linkage of *RPB1* with a morphological marker was detected by a deviation from independent segregation in the *F₂* population that was calculated by a χ²-test for independence. Recombination fraction was estimated by the product ratio method using the table of Immer (1930).

For all genetic experiments ecotype Cvi-0 was inoculated as the susceptible control and only tests in which Cvi-0 was scored 100% susceptible were considered for genetic analysis.

ACKNOWLEDGMENTS

We thank Elke Rohls for technical assistance and Hubertus Kohn for critical reading of the manuscript. This work was supported by the Bundesministerium für Forschung und Technologie.

LITERATURE CITED


