Map Positions of Three Loci in *Arabidopsis thaliana* Associated with Isolate-Specific Recognition of *Peronospora parasitica* (Downy Mildew)

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Our research is aimed at understanding the molecular basis for gene-for-gene interactions between plant parasites and their hosts. As a prelude to cloning, the positions in the *Arabidopsis thaliana* genome were investigated for four of the 10 loci (*RPP1, RPP2, RPP4,* and *RPP7*) that have been identified as associated with the genotype-specific recognition of the biotrophic oomycete *Peronospora parasitica* (downy mildew). A single cross between accessions Col-5 and Nd-1 was primarily used to map their chromosomal locations. *RPP1* from Nd-1 was characterized by the absence of asexual sporulation and the occurrence of necrotic pits, visible macroscopically on cotyledons 3 days after inoculation with isolates *Emoy2* or *Hiks1*; this locus was mapped to chromosome 3 in the interval between *gl1* and m249 (6.6 cM above m249 and 3.9 cM below a RAPD marker $OPC12_{1250}$). *RPP2* and *RPP7* from accession Col-5(*gl1*) were characterized by the absence of asexual sporulation and the occurrence of necrotic flecks visible 7 days after inoculation with isolates *Cala2* and *Hiks1,* respectively. *RPP2* was located between *ag* and B9 on chromosome 4. *RPP7* maps within 13 cM of m422, but linkage with other markers on chromosome 5 was not confirmed. *RPP4* from accession Col-5 was characterized by the occurrence of necrotic flecks and delayed, light sporulation 7 days after inoculation with isolates *Emoy2* and *Emwa1*; this locus also maps to chromosome 4, 14.8 cM above *RPP2.*

Additional keywords: avirulence, disease resistance, resistance genes.

The molecular basis for the ability of plants to discriminate among different genotypes of a potential parasite remains one of the greatest unsolved mysteries in plant science. It is clear that plants produce a diversity of gene products following challenge by potential parasites and that the dynamics and detail of these responses differ depending on the genetic identity of the parasite and the host (Collinge and Slusarenko 1987). It has proved difficult to establish with certainty, however, direct causal relationships between these response gene products and the manifestation of resistance. It is generally accepted that the genotype specificity of a plant's response resides in genes for recognition in both partners (resistance/susceptibility genes in the host and virulence/avirulence genes in the parasite). The products of these genes are believed to interact in a highly specific manner. This relationship was first formalized by Flor (1955, 1971) as the so-called "gene-for-gene" hypothesis, following studies with flax and flax rust. Since then, gene-for-gene relationships have been shown to govern the variation observed in many host-parasite associations (Ellingboe 1981, 1984; Crute 1985; Keen 1982, 1990).

It has proved relatively straightforward to isolate so-called avirulence (*avr*) genes from bacterial pathogens (Keen and Staskawicz 1988; Keen 1990; de Wit 1992) and, hence, to determine their DNA sequences and primary protein products. Avirulence genes studied to date appear to have little relationship to one another, and no obvious conclusions can yet be drawn about any common molecular mechanism by which plants detect and respond to their presence. Movement of *avr* genes between different bacteria has shown that plants can carry genes capable of recognizing *avr* gene products isolated from bacteria not considered as pathogens of the species (Whalen *et al.* 1988; Fillingham *et al.* 1992; Dangl *et al.* 1992b). This implies that plants carry genes that recognize a diversity of pathogen gene products and that only through a molecular analysis of a similar diversity of plant genes will an understanding of gene-for-gene specificity be achieved.

Genes involved in the recognition of parasites are discriminated through their specific interaction with particular parasite isolates, by characteristic phenotypes associated with particular combinations of host and isolate, and following classical genetic analyses of segregating progenies generated from crosses between different host genotypes. The development of molecular mapping techniques has provided the opportunity to isolate such genes using position-based and chromosome-walking strategies. Thus, efforts to isolate genes for recognition of *Fulvia fulvum* and *Pseudomonas syringae* pv. *tomato* (*Pst*) in tomato and of *Bremia lactucae* in lettuce have been in progress for several years (Dickinson *et al.* 1993; Jones *et*

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al. 1993; Martin *et al.* 1993a; Michelmore *et al.* 1991, 1993). The successful cloning of the *Pto* allele from tomato for recognition *Pst* appears to be close (Martin *et al.* 1993b). The identification of suitable pathosystems in *Arabidopsis thaliana* (L.) Heynh. now provides the opportunity to utilize the considerable advantages of this plant species to isolate and study genes for recognition of several different types of parasite (Meyerowitz 1989; Koch and Slusarenko 1990a, 1990b; Davis *et al.* 1991; Debener 1991; Simons *et al.* 1991; Tsuji *et al.* 1991; Whalen *et al.* 1991; Dangl *et al.* 1992a; Parker *et al.* 1993a).

Debener *et al.* (1991) have reported the precise map position, on the upper arm of chromosome 3, of a gene (*RPM1*) in *A. thaliana* for recognition of the bacterial parasite *P. s.* pv. *maculicula*. More recently, Kunkel *et al.* (1993) have shown that a gene (*RPT2*) controlling resistance to *P. s.* pv. *tomato* carrying the cloned avirulence gene *avr*Rpt2 is located on chromosome 4 of *A. thaliana*, 1 cM from the locus defined by RFLP probe m600. These observations suggest that genes involved in specific recognition of parasites exist at several locations in the *A. thaliana* genome.

A companion paper (Holub *et al.* 1994) describes the phenotypic and genotypic variation that exists in interactions between *A. thaliana* and *P. parasitica*. By choosing an appropriate cross between two accessions, it was proposed that several host loci, associated with different interaction phenotypes, could be investigated following inoculation with several parasite isolates. Molecular analysis and comparison of genes associated with different phenotypes will improve the prospects of being able to dissect the molecular mechanisms underlying genotype-specific recognition. This paper describes the commencement of a program to isolate genes from *A. thaliana* for the genotype-specific recognition of *Peronospora parasitica*, an obligately biotrophic oomycete. We describe how a cross between accessions Col-5 and Nd-1 was used to map four recognition loci in *A. thaliana* including examples associated with phenotypes differing in the extent of host cell necrosis and the timing and degree of sporulation by *P. parasitica*. Two of these loci have been subject to fine-scale mapping.

RESULTS

Interaction phenotypes and genetic loci.

Holub *et al.* (1994) described four different phenotypes among interactions between *A. thaliana* accessions Col-5 and Nd-1 following inoculation with *P. parasitica* isolates *Cala2*, *Emoy2*, and *Hiks1*, and they postulated the existence of *RPP* loci (recognition of *P. parasitica*) associated with each phenotype. The interaction between Nd-1 and *Cala2* was defined as fully susceptible (compatible). In this combination, asexual reproduction by the parasite was first evident 3 dai (days after inoculation) becoming profuse 7 dai; there was no macroscopic evidence of any host response (Table 1 and Fig. 1). The interaction between Nd-1 and either *Emoy2* or *Hiks1* was characterized by the lack of asexual sporulation by the parasite and the occurrence of extensive host cell necrosis, visible as necrotic pits 3 dai. A single-host locus *RPP1* was postulated to be associated with this phenotype. The interaction between Col-5 and *Cala2* was characterized by the lack of asexual sporulation by the parasite and the occurrence of minute necrotic flecks visible 7 dai; *RPP2* was postulated as the host locus associated with this interaction phenotype. A similar interaction phenotype was observed between Col-5 and *Hiks1*, but a different host locus, *RPP7*, was postulated. Finally, the interaction between Col-5 and *Emoy2* was characterized by

Table 1. Phenotypic description and genotypic model of interactions between five isolates of *Peronospora parasitica* and four accessions of *Arabidopsis thaliana*

						P. parasitica isolate				
						***ATR*[a]**	***Emoy2***	***Emwa1***	***Cala2***	***Hiks1***	***Wela3***
						1	1	0	0	√	0
						2	0	0	1	0	0
						4	1	√	0	0	0
						6	0	0	0	0	1
						7	0	0	0	1	0
A. thaliana	***RPP*[a]**										
Genotype	1	2	4	6	7						
Nd-1	1	0	0	0	0		PN[b] (1)	DH	EH	PN (1)	DM
Col-5	0	1	1	1	1		FDL (4)	FR (4)	FR (2)	FR (7)	FR (6)
Ksk-1	0	√	0	0	0		FN (?)	FN (?)	FR (2)	EH	FN (?)
Wei-1	0	0	0	0	0		DL	DL	DL	DL	EH

[a] *RPP* = recognition of *P. parasitica*; and *ATR* = *A. thaliana* recognized. The *RPP* columns 1, 2, 4, 6 and 7 represent the loci that have been identified genetically in *A. thaliana*; alleles at each locus are designated below for each accession. Numbers for *ATR* loci of *P. parasitica* are inferred from a theoretical gene-for-gene relationship and thus correspond with numbers for *RPP* loci. Alleles within each *RPP* column or *ATR* row are indicated as follows: 1 = the matching alleles associated with an incompatible interaction and used to define the corresponding loci, √ = alleles determined by genetic analysis of the host to reside at a previously identified locus, and 0 = alleles associated with full compatibility (i.e., do not contribute to recognition in the combinations shown).

[b] Interaction phenotypes were characterized using an assessment of host and parasite characteristics: the emergence of sporangiophores of *P. parasitica* as early (E) 3 days after inoculation (dai) or delayed (D) >4 dai; the intensity of asexual sporulation as heavy (H; >20 sporangiophores per cotyledon), light (L; <10 sporangiophores), rare (R; <5 sporangiophores on <10% of inocuated seedlings) or none (N); and the type of response by *A. thaliana* as minute necrotic flecks (F) evident 7 dai, or necrotic pits (P) observed as early as 3 dai and often expanding until much of cotyledon is necrotic 7 dai. The *RPP-ATR* gene pair associated with a given interaction phenotype is shown in parentheses. ? = gene pair not characterized. No gene pair is shown for EH, DL, DM, and DH phenotypes.

delayed, light sporulation by the parasite and a host response involving minute necrotic flecks visible 7 dai. *RPP4* was postulated as the host locus associated with this phenotype.

Predicting genotypes at *RPP* loci in an F_2 population of *A. thaliana*.

F_3 families from a cross between Col-5 and Nd-1 were used to predict the genotype at *RPP* loci of the original F_2 parents following inoculations with *P. parasitica* isolates *Emoy2, Emwa1, Cala2, Hiks1,* and *Wela3*. Figure 2 provides examples of the way in which this was achieved (for further details see Holub *et al.* 1994). From F_2 analysis we were predicting segregation of a single gene for recognition of each isolate and, hence, the F_3 analysis could be carried out on a relatively small number of plants. However, critical families in the mapping of the loci were rescored for their resistance phenotype. Phenotypes were assigned to one of four classes: no asexual sporulation exhibited by the parasite with a host response of necrotic pits; no sporulation and any host response other than necrotic pits (none, flecks, or cavities); delayed and light sporulation evident 7 dai with or without necrotic flecks; and sporulation first evident 3 dai becoming profuse without an obvious host response.

The segregation of alleles at locus *RPP1* was unambiguous. An F_2 individual that was homozygous for the Col-5 allele was predicted if none of the F_3 progeny exhibited necrotic pits following inoculation with *Emoy2,* whereas an F_2 individual homozygous for the Nd-1 allele was predicted if all of the F_3 progeny exhibited pits. When F_3 progeny segregated for the occurrence of pits, *RPP1* was shown at F_2 to be in the heterozygous condition. All 123 families segregated similarly following inoculation with *Emoy2* and *Hiks1,* and it is likely that the Nd-1 allele at *RPP1* is involved in the recognition of both isolates (Holub *et al.* 1994). Although Nd-1 may carry two alleles at closely linked loci, only a single allele was named, referred to as *RPP1-1*. Segregation of alleles at *RPP2* was also unambiguous following inoculation with isolate *Cala2*. The allele from Col-5 at this locus is referred to as *RPP2-1*.

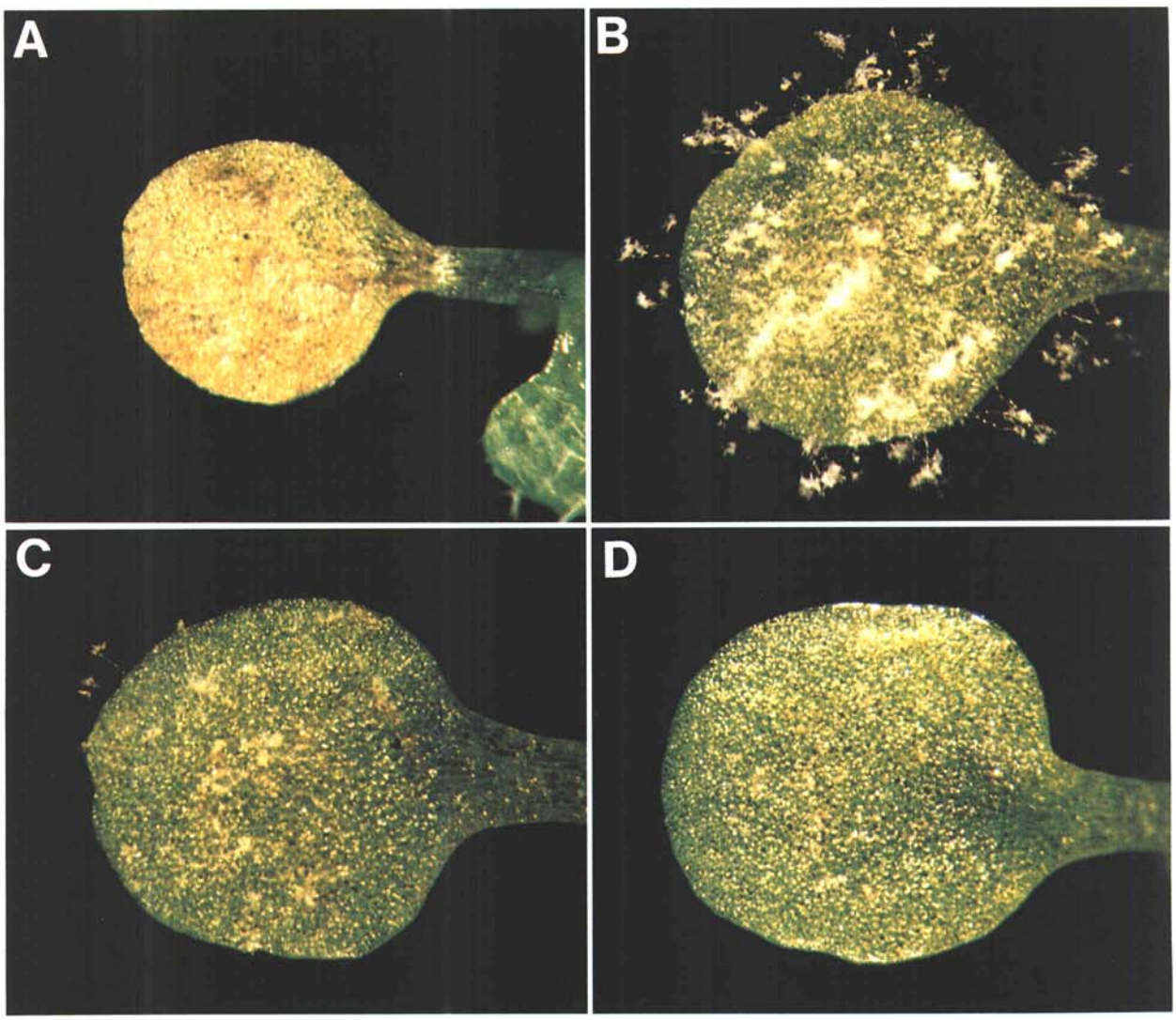
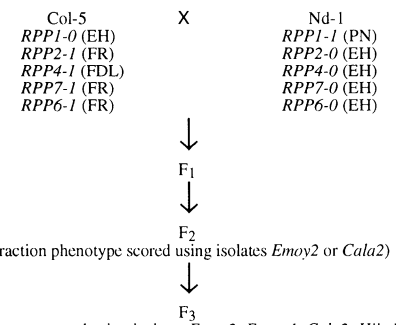


Fig. 1. Variation for interaction phenotype among combinations between accessions of *Arabidopsis thaliana* and isolates of *Peronospora parasitica* macroscopically 7 days after inoculation. **A,** No sporulation with uniformly colored, fully expanded pits (Nd-1 vs. *Emoy2*); **B,** early and heavy asexual sporulation (Nd-1 vs. *Cala2*); **C,** delayed and sparse sporulation with necrotic flecks (Col-5 vs. *Emoy2*); **D,** no sporulation with necrotic flecks (Col-5 vs. *Cala2*).

The hyperstatic phenotype (necrotic pits) associated with *RPP1-1* (Holub *et al.* 1994) posed a complication in determining genotypes at *RPP4* and *RPP7*. It was not possible to determine the genotype of these latter two loci following inoculation with *Emoy2* or *Hiks1*, respectively, if F_3 progeny were homozygous for *RPP1-1*. However, in the absence of this allele, segregation at *RPP4* was clearly evident following inoculation with *Emoy2* (e.g., F_3 families 1, 2, and 3, Fig. 2). In addition, the effect of *RPP1-1* ceased to be a complicating factor when a key diagnostic isolate, *Emwa1*, was used (Holub *et al.* 1994). This isolate was used to determine the genotype at *RPP4* in F_3 families that otherwise exhibited pits when inoculated with *Emoy2*. The allele from Col-5 at this locus is referred to as *RPP4-1*. Only isolate *Hiks1* was available for determining segregation at *RPP7*, hence the genotype at *RPP7* for F_3 families homozygous for *RPP1-1* is unknown. The allele from Col-5 at this locus is referred to as *RPP7-1*.

In summary, inoculations with four isolates can be used to identify three recognition alleles from Col-5 (*RPP2-1; RPP4-1*, and *RPP7-1*) and one from Nd-1 (*RPP1-1*). An additional isolate, *Wela3*, can be used to distinguish a fifth locus (*RPP6-1*) in Col-5 associated with the occurrence of necrotic flecks and rare sporulation (Mauch-Mani *et al.* 1993; Holub *et al.* 1994). It should be possible to map all five loci in this single cross, since it was possible to predict the genotype of most F_2 individuals from the segregation of F_3 progeny.



Illustrative sample of F_3 segregation ratios used to predict F_2 genotype at *RPP* loci

F_3	F_3 phenotypic segregation[a]					Predicted F_2 genotype[b]				
family	Emoy2	Emwa1	Cala2	Wela3	Hiks1	R1	R2	R4[c]	R6	R7
911531	0:0:0:11	0:1:0:16	0:0:1:14	0:0:4:7	0:21:6:13	11	22	22	22	12
911576	0:14:6:0	0:24:0:0	0:28:0:0	0:10:2:0	0:14:4:0	11	11	11	12	11
911534	0:10:2:3	0:17:4:1	0:15:2:1	0:7:2:2	0:15:4:3	11	12	12	12	12
911554	16:0:0:0	0:3:3:16	0:0:0:26	0:0:4:7	48:1:0:0	22	22	22	22	?
911463	29:1:0:2	0:1:0:24	0:0:0:11	0:8:2:0	13:0:0:6	12	22	22	12	22
911433	8:0:2:0	0:16:5:1	0:13:6:3	0:9:0:0	24:3:3:3	12	12	12	11	12
Col-5	0:60:39:3	0:136:0:0	0:45:6:0	0:49:0:0	0:21:5:0	11	11	11	11	11
Nd-1	167:4:0:0	0:18:25:86	0:1:16:40	0:6:36:27	26:0:0:0	22	22	22	22	22

[a] Necrotic pits with no asexual sporulation : no pits and no sporulation : no pits and delayed, light sporulation : early and heavy sporulation.

[b] 11 = homozygous for Col-5 allele; 22 = homozygous for Nd-1 allele; and 12 = heterozygous.

[c] The genotype at *RPP4* could not be determined following inoculation with *Emoy2* due to necrotic pits associated with *RPP1-1*. However, the 22 genotype was predicted when a segregation of 0:0:0:12 was observed following inoculation with *Emwa1*, an isolate which is recognized by *RPP4-1* but sporulates freely on Nd-0. Similarly, the genotype at *RPP7* could not be determined following inoculation with *Hiks1* due to necrotic pits associated with *RPP1-1*. No alternative isolate was available for predicting the genotype at *RPP7*.

Fig. 2. Segregation for recognition alleles and predicted F2 genotypes for *RPP1, RPP2, RPP4,* and *RPP7* among F3 families of the cross between Col-5 and Nd-1.

Map positions of recognition loci.

The DNA from more than 100 F_3 families was used to map the location of *RPP* loci relative to molecular markers. Initially, we relied upon the least ambiguous class of homozygous susceptible families to coarsely map the position of each locus. Subsequently, as linked molecular markers were identified, confirmation of map position was sought using heterozygous and homozygous resistant families. Linkage was observed between loci *RPP1* and *GL-1* (the recessive allele carried by Col-5 [*gl1*] exhibits a glabrous phenotype). Thus, *RPP1* was located to chromosome 3 at a distance of about 17 cM from *GL-1*. Segregation of RFLP loci revealed by probes m249, m576, and m460 confirmed the location of *RPP1* at a position below *GL-1*. The RFLP locus m249 mapped 6.6 cM below *RPP1*. An illustrative sample of the data used to map the location of *RPP1* relative to other loci is shown in Table 2. The interval containing *RPP1* between *GL-1* and m249 is about 21.3 cM.

Because no further probes were available identifying RFLP loci within the interval containing *RPP1*, RAPD loci were sought for this region of the genome. Individual F_3 families were identified that were homozygous for either *RPP1-1* or *RPP1-0* (the corresponding Col-5 locus at which recognition of *Emoy2* does not occur) and were also homozygous for the flanking RFLP loci derived from the same parent. This effectively resulted in an enrichment for DNA surrounding the recognition locus from each single parent. DNA from these families was combined into two pools and screened for the occurrence of diagnostic RAPDs. More than 200 primers were tested and three were found to amplify bands linked to *RPP1* (Table 2). $OPC12_{1250}$ and $OPA02_{600}$ both amplified specific loci in the resistant pool and OPH12 amplified a specific locus in the susceptible pool. $OPC12_{1250}$ mapped 3.9 cM above *RPP1*; $OPA02_{600}$ 4.2 cM above $OPC12_{1250}$, and 6.6 cM below *GL1*. Although the locus amplified by OPH12 mapped between *GL1* and *RPP1-1* it was not pursued further because it could not be positioned relative to $OPC12_{1250}$ and $OPA02_{600}$ due to the dominant nature of the marker. This exercise defined a narrower interval of 10.5 cM spanning the *RPP1* locus (see Fig. 3). Current efforts to define the position of this locus

Table 2. Segregation of loci among F_3 families of Col-5 × Nd-1 that were critical to the mapping of *RPP1*

	Interval of loci on chromosome 3[a]					
F_3 family	*GL1*	$OPA02_{600}$	$OPC12_{1250}$	*RPP1*	m249	m576
1449	12	11	11	11	11	11
1450	11	2	11	11	11	11
1439	11	2	11	11	11	11
1442	NT	2	2	11	11	11
1522	12	2	2	11	11	12
1532	NT	2	2	11	11	11
1568	NT	2	2	11	11	NT
1431	11	11	11	11	12	12
1501	NT	11	11	11	12	12
1608	NT	11	11	11	12	12
1505	12	11	11	11	11	12
1469	22	2	2	22	22	12
1432	NT	2	2	12	22	22

[a] 11 = homozygous for Col-5 allele; 22 = homozygous for Nd-1 allele; 12 = heterozygous; NT = not tested. m249 and m576 are RFLP loci; $OPA02_{600}$ and $OPC12_{1250}$ are RAPD loci for the completely dominant alleles from Nd-1; and *GL1* is a locus for the glabrous phenotype.

more precisely include attempts to determine the physical distance between m249 and $OPC12_{1250}$, the identification of further markers within the interval and the identification of YAC clones that hybridize to the flanking markers. The latter may allow new RFLP loci to be identified as a prelude to embarking on a chromosome walk to *RPP1*.

RPP2 was not linked to *RPP1* (49% recombination), hence it was necessary to search for the locus using evenly spaced RFLP loci in a systematic manner. In this way *RPP2* was located on chromosome 4 (Fig. 3). *RPP2* was located within an interval that was defined between the RFLP loci identified by probes m210 and m600. Initial mapping data suggested that *RPP2-1* was 17.9 cM above m600 and 21.5 cM below m326, positioning it in the region of the RFLP locus identified by probe m557 (Table 3). Although a range of probes identify RFLP loci in this region of chromosome 4 in the cross between Col-5 (*gl1*) and Nd-1, few were dimorphic with our standard set of mapping enzymes. Those probes that did result in dimorphic banding patterns enabled *RPP2-1* to be located 4.8 cM below *Agamous(ag)* and 9.1 cM above the locus defined by probe B9. Current efforts are focused on more precisely defining the interval containing *RPP2-1* by utilizing end probes generated from YACs that map to this area (R. Schmidt and Caroline Dean, personal communication) to detect further polymorphisms.

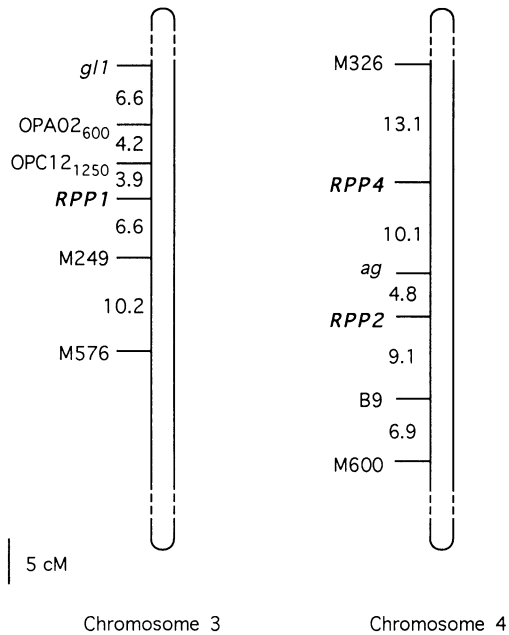


Fig. 3. Map location of *RPP1*, *RPP2*, and *RPP4*.

Holub *et al.* (1994) postulated that accession Ksk-1 carries *RPP2-2*, a potential allele of *RPP2-1*, which recognizes *Cala2*. In the F_2 generation of a cross between Col-5 and Ksk-1 following inoculation with *Cala2*, none of the 141 progeny tested exhibited early, profuse sporulation. This was interpreted as evidence that both accessions carried alleles at *RPP2* or at closely linked loci. In addition, the response of Ksk-1 following inoculation with *Cala2* is associated with the occurrence of necrotic flecks similar to Col-5 (Table 1). Probes identifying RFLPs in the interval between m210 and m600 detected dimorphisms between Wei-1 and Ksk-1 (probes m210, m580, B9, and *ag* revealed polymorphisms among *Eco*R1-digested genomic DNA and probes g4564, g4539, and m600 revealed polymorphisms among *Cla*I digested genomic DNA). Families of the cross were scored for the necrotic flecks characteristic of Ksk-1 at 7 dai when the Wei-1 control seedlings showed sporulation. Segregation was studied among 43 F_3 families, and it was shown that the locus revealed by probe m557 was above *RPP2*; this defined an interval between m557 and m600 within which *RPP2* was located. Data for the critical recombinant families (Table 3) suggested a location for *RPP2-2* that was 7.1 cM below the locus *ag* but 7.8 cM above the locus revealed by probe B9. Because there were too few F_3 families available to obtain a precise map position, further analysis of this locus will be carried out using 106 recombinant inbreds (F_6) of Wei-1 × Ksk-1.

Holub *et al.* (1994) suggested that *RPP4* and *RPP2* were linked loci. By using isolate *Emwa1* to determine the genotype at *RPP4* for F_2 individuals from Col-5 × Nd-1, *RPP4-1* was found to lie 10.1 cM above *ag* and 13.1cM below the locus defined by probe m326 (Table3; Fig. 3).

Table 3. Segregation of loci among F_3 families of Col-5 × Nd-1 and Wei-1 × Ksk-1 that were critical to the mapping of *RPP2* and *RPP4*

A. thaliana Cross	F_3 family	Interval of loci on chromosome 4[a]							
		m326	g4539	*RPP4*	m557	*Ag*	*RPP2*	B9	m600
Col-5 × Nd-1	1430	12	ND	12	ND	22	22	22	22
	1608	12	ND	12	ND	22	22	22	22
	1567	22	ND	12	ND	12	12	12	12
	1436	12	ND	22	ND	22	22	22	22
	1461	12	ND	22	ND	22	22	22	NT
	1435	11	ND	11	ND	12	12	22	22
	1565	22	ND	11	ND	11	22	12	12
Wei-1 × Ksk-1	2047	NT	11	NS	11	11	11	12	12
	2247	NT	11	NS	11	11	11	12	12
	2310	NT	11	NS	11	11	11	12	12
	2275	NT	11	NS	11	11	11	11	12
	2250	NT	11	NS	11	11	11	11	12
	2055	NT	11	NS	11	11	12	12	12
	2060	NT	11	NS	11	11	12	12	12
	2057	NT	12	NS	12	12	12	12	11
	2058	NT	22	NS	22	22	22	12	12

[a] 11 = homozygous for female allele (Col-5 or Wei-1); 22 = homozygous for male allele (Nd-1 or Ksk-1); 12 = heterozygous; ND = no dimorphism found between parents of the cross using a standard set of enzymes; NS = no segregation at the locus in the cross; and NT = not tested. m326, g4539, m557, *Ag*, B9, and m600 are RFLP loci.

Cosegregation studies suggested that *RPP7* was not linked to *RPP1* (47% recombination) or *RPP4* (49% recombination) (Holub *et al.* 1994) and this has been confirmed using probes that reveal linked RFLP loci. However, possible linkage between *RPP7* and *RPP2* (34% recombination) could not be ruled out. The locus revealed by probe m600, which maps approximately 17.9 cM below *RPP2*, also showed only 39% recombination with *RPP7*. If *RPP7* is located below *RPP2* on chromosome 4, the data for the segregation of m600 would have indicated closer linkage with *RPP7* than with *RPP2* and this was not the case. Recent data have demonstrated linkage (approximately 13 cM) of *RPP7* to a locus revealed by probe m422. However, extensive mapping data suggest that other RFLP loci (m247, m423, and g2105), previously reported to be closely linked to m422, show no linkage to either *RPP7* or m422. We are currently checking that our probe m422 is the same as that mapped by Chang *et al.* (1988) to chromosome 5, attempting to find linkage to other markers (currently no linkage has been found, but the bottom of chromosome 1, the top of chromosome 3, and all of chromosome 2 are essentially unmapped) and identifying YAC clones using m422 as a probe in order to produce further markers that will confirm linkage to *RPP7-1*. *RPP7-1* is associated with a similar interaction phenotype to *RPP2-1* and *RPP2-2* and because it appears to map at a different chromosomal location it is of continuing interest (Table 1).

It should also be possible to map a further locus (*RPP6*) using the same cross (Table 1). To do this, families inoculated with isolate *Wela3* were scored 7 dai when Nd-1 control seedlings showed sporulation. From data currently available this locus does not appear to be linked to other *RPP* loci or molecular markers so far analyzed (Table 4).

DISCUSSION

Elucidation of the role of recognition genes in the defense of plants against fungal parasites is important to progress understanding of the mechanisms of disease resistance in plants. The work we present here represents the foundation in our attempts to understand the molecular mechanisms of gene-for-gene recognition.

Holub *et al.* (1994) have postulated the occurrence of 12 gene pairs involved in the gene-for-gene interaction between the oomycete parasite *P. parasitica* and *A. thaliana*. The interaction phenotype varied depending on parasite isolate and host locus (Table 1). Inoculation of accession Nd-1 with isolates *Emoy2* and *Hiks1* resulted in the occurrence of necrotic pits and the complete absence of sporulation. When the same accession was inoculated with isolate *Cala2* the parasite sporulated profusely. However, inoculation of accession Col-5 with isolates *Hiks1* or *Cala2* resulted in necrotic flecks, again in the absence of sporulation. In contrast, when accession Col-5 was

Table 4. Percentage recombination between *RPP* loci and markers on the *Arabidopsis* genome[a]

		RPP1		*RPP2*		*RPP4*		*RPP6*		RPP7	
Chr.	**Locus**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**	**n**	**%**
1	m322	10	45	10	30	12	21	11	27	5	20
	m219	23	59	23	30	25	30	24	33	15	50
	m201	27	59	27	35	29	38	28	45	18	31
	m335	69	49	69	38	71	36	70	37	51	32
2	m246	9	50	9	44	11	32	10	25	6	17
3	GL1	44	20	44	52	44	49	43	36	35	53
	RPP1	96	...	96	51	94	44	95	44	76	59
	m249	73	6	72	47	74	41	74	41	55	47
	m576	63	16	62	48	64	42	64	38	45	47
	m460	23	24	23	46	25	32	24	44	19	53
4	m210	11	36	11	32	13	27	12	46	6	50
	m580	30	42	30	17	32	13	31	47	19	42
	m326	53	53	53	14	55	15	54	42	39	33
	RPP4	94	44	93	15	96	...	94	39	73	48
	m557	36	50	36	6	38	13	37	45	24	35
	AG1	39	49	39	3	41	12	40	44	28	38
	RPP2	96	51	96	...	93	15	95	41	76	39
	B9	39	47	39	6	41	13	40	40	28	39
	m600	62	55	62	12	64	22	63	43	46	35
	m272	42	57	42	13	44	19	43	48	31	44
	m214	28	41	28	16	30	23	29	40	17	32
5	m224	42	46	42	48	44	43	43	30	31	42
	m247	74	41	74	46	74	43	75	40	57	39
	m423	81	41	81	43	81	43	82	37	62	42
	g2105	73	36	73	38	73	36	74	39	61	44
	m331	38	41	38	50	40	45	39	31	25	38
	m211	38	50	38	36	40	38	39	33	27	35
?	*RPP6*	95	44	95	41	94	39	96	...	75	33
	RPP7	76	59	76	39	73	48	75	33	76	...
	m422	81	53	81	43	80	47	82	35	64	13

[a] These data were obtained from a set of 96 F_3 families following inoculation with five isolates of *P. parasitica*. The families represented a subset of those used to fine-scale map the *RPP* loci. n represents the number of F_3 families tested; % represents the percentage recombination between the marker locus and the *RPP* locus. Chi-squared values were not calculated because the set of 96 F_3 families used here were preselected for homozygous susceptibility to either *Emoy2* or *Cala2* before testing with other isolates. Such families were unambiguous for purposes of mapping but were consequently nonrandom for regions of chromosomes 3 and 4.

inoculated with isolate *Emoy2*, sporulation occurred, but this was delayed and light compared to other combinations of isolate and accession considered to be fully compatible (e.g., Nd-1 and *Cala2*). Hence, with these two host accessions and three parasite isolates, it was possible to identify different interaction phenotypes. In parallel with the formal genetic analysis reported by Holub *et al.* (1994), available molecular polymorphisms, already mapped in the *A. thaliana* genome, were used to determine the chromosomal location of loci controlling isolate specific variation for interaction phenotypes.

Using a single cross between *A. thaliana* accessions Col-5 and Nd-1, it has been possible to map the genomic location of three loci (*RPP1*, *RPP2*, and *RPP4*) involved in specific recognition of three isolates of *P. parasitica*. *RPP1*, associated with the occurrence of necrotic pits, maps to chromosome 3 between the loci defined by markers m249 and $OPC12_{1250}$. It has not yet been possible to demonstrate whether the response of Nd-1 to isolates *Emoy2* and *Hiks1* (both associated with necrotic pits) is due to alleles at two closely linked loci, the same allele at a single locus, or different alleles at a single locus. However, the loci or locus controlling the pitting response to both isolates map to the same locus on chromosome 3. One way of demonstrating the existence of different alleles for the recognition of each isolate will be to produce recombinants in the pathogen from a cross between isolates *Emoy2* and *Hiks1*.

The necrotic flecking response observed following the inoculation of accession Col-5 with isolates *Hiks1* and *Cala2* are controlled by genes that map to separate loci (*RPP7* and *RPP2,* respectively). *RPP2* maps to the lower arm of chromosome 4 closely linked with the locus *Ag*. The location of *RPP7* has yet to be defined but shows no linkage to the other *RPP* loci. This provides an example of two different *RPP* loci associated with similar interaction phenotypes. The cloning of these genes will enable us to discover whether they are the result of gene duplication and translocation or have evolved separately and developed a function that results in a common phenotype. Additionally, we have identified a second allele of *RPP2* (*RPP2-2*) in accession Ksk-1 that maps to the same interval as *RPP2-1*. Cloning of *RPP2-1* from Col-5 may then enable us to obtain *RPP2-2* rapidly from Ksk-1 by way of homologous probing.

The locus *RPP4*, associated with the delayed, light sporulation observed following the inoculation of Col-5 with *Emoy2* also maps to chromosome 4 in the region of *RPP2*. An isolate of the parasite (*Emwa1*), diagnostic for *RPP4* or a closely linked locus in Col-5, allowed the location of *RPP4* to be precisely determined, and it lies between m326 and *Ag*. The location of *RPP4* is likely to be in the region of *RPP5* (Holub *et al.* 1994; Parker *et al.* 1993b) a locus identified by the presence of allele of *RPP5-1* in La-*er* that is associated with lack of sporulation and the occurrence of necrotic flecks following inoculation with isolate *Noco2*. Therefore, *RPP2*, *RPP5*, and *RPP4* provide an example of linked loci associated with the expression of different yet quite similar interaction phenotypes. Comparisons between the sequences of alleles at such loci will provide insights into the molecular mechanisms of specific recognition and the processes that lead to variation in interaction phenotype.

The locus *RPP7*, associated with necrotic flecks and rare, light sporulation following inoculation of Col-5 with *Hiks1* appears to be linked to a polymorphism detected by probe m422 that had previously been mapped to chromosome 5 (Chang *et al.* 1988; Cherry *et al.* 1992). However, recent data suggest that the published flanking loci do not show linkage to *RPP7* or m422. *RPP7* is an important locus as it suggests that there are other regions of the genome involved in the isolate-specific recognition of *P. parasitica*.

This study has shown that it is possible to identify and map single loci in *A. thaliana* involved in the isolate-specific recognition of *P. parasitica*. *A. thaliana* accessions carrying different recognition alleles at these loci express a range of interaction phenotypes in combination with different parasite isolates. These loci map to several chromosome locations and some appear to be clustered (e.g., on chromosome 4). The map locations have been firmly established for three recognition loci (*RPP1*, *RPP2*, and *RPP4*), and we are now attempting to clone alleles at these loci by using a map-based approach utilizing the cross between Col-5 and Nd-1. Further loci will, undoubtedly, be localized in the genome of *A. thaliana* as new diagnostic pathogen isolates are identified. The mapping of these loci will be aided by using the map locations reported here as a framework from which to choose markers that have a higher probability of being linked to loci involved in isolate-specific recognition of *P. parasitica*. A comparison of primary sequence information from alleles at these and other loci, and from similar alleles present in other accessions, may allow us to understand their evolution and enable us to associate a function with the gene products. Such an analysis is the first step towards elucidating the molecular mechanisms involved in gene-for-gene recognition.

MATERIALS AND METHODS

Accessions of *A. thaliana* and isolates of *P. parasitica*.

The origins of *A. thaliana* accessions (Col-5, Nd-1, Ksk-1, and Wei-1) and *P. parasitica* isolates (*Cala2, Emoy2, Hiks1, Emwa 1,* and *Wela3*) were described by Holub *et al.* (1994). Methods for culturing the isolates, preparation of inoculum, and determination of interaction phenotypes have been described previously (Dangl *et al.* 1992a; Holub *et al.* 1994).

Isolation of plant DNA.

Seeds were surfaced sterilized in microfuge tubes by washing them in 70% ethanol for 1 min, followed by washing in 3% sodium hypochlorite for 3 min, and then rinsed four times with sterile water. Twenty to 50 surface-sterilized seeds from each F_3 family were then added to a 500-ml conical flask containing 125 ml of MS media (1× MS, 3% sucrose, 0.5g L^{-1} MES buffer, pH 5.8; Murashige and Skoog 1962) and grown in continuous dark on a rotary shaker (80 rpm) at 25° C. Plant tissues were harvested after 3 wk, frozen in liquid N_2, and stored at –80° C until required. DNA was isolated according to the method of Dellaporta *et al.* (1983).

RFLP analysis.

Restriction digests, Southern blotting, and hybridization of labeled probes to isolated plant genomic DNA was carried out using standard techniques (Ausubel *et al.* 1990). RFLP probes were either lambda clones (Chang *et al.* 1988; provided by J. Dangl, Cologne, Germany; designated m) or cosmid clones (Nam *et al.* 1989; provided by R. Schmidt and C.

Dean, Norwich, UK: designated g). Probe B9 was kindly provided by G. Coupland, Norwich, UK, and the *agamous* probe by M. Yanofsky, University of California, San Diego. Probes were labeled using the Pharmacia Oligolabeling system according to the manufacturer's instructions. The following standard set of restriction enzymes were used to identify polymorphisms between accessions of *Arabidopsis*: *Eco*RI, *Bam*HI, *Bgl*II, *Cla*I, *Eco*RV, *Hin*DIII, *Pst*I, *Sst*I, *Xba*I, *Xho*I, and *Sau*3AI.

RAPD analysis.

Bulk segregant analysis (Michelmore *et al.* 1991) was used to identify random amplified polymorphic DNA (RAPD) markers located near to resistance loci. Two pools were created each containing DNA from 10 F_3 families that were either homozygous susceptible or resistant at the locus under investigation and also at the two nearest flanking markers previously identified for each parental type. DNA samples were quantified on aga rose gels and approximately 2.5 µg of each DNA was added to the pool. The mixture was then diluted to a final concentration of 5 ng/µl. Random oligonucleotide primers for RAPD analysis were obtained from Operon Technologies (Alameda, CA). Amplification reactions were carried out in 96-well polycarbonate microtiter dishes at a final volume of 25 µl on a Techne PHC-3 thermocycler (Cambridge, U.K.). Amplification reactions contained 10 mM Tris-HCl (pH 9.0), 50 mM KCl, 1% Triton X-100, 2.5 mM $MgCl_2$, 200 µM of each dNTP (Pharmacia), 0.4 µM RAPD primer, 1 unit Taq polymerase (Promega), and 25 ng of genomic DNA. The reaction was overlaid with 20 µl of mineral oil. The following reaction conditions were then applied: 1) 95° C for 4 min × 1 cycle; 2) 94° C for 1 min, 35° C for 1 min, 74° C for 2 min × 45 cycles; 3) 74° C for 10 min × 1 cycle; and 4) 4° C until ready to analyze. Amplification products were visualized by electrophoresis in 2% agarose gels (New Brunswick Scientific) followed by staining with ethidium bromide.

Primers that amplified a band in only one of the two pools were subsequently used to investigate each individual that comprised the pool. If the banding pattern was confirmed, the segregation of the RAPD marker was studied in the F_3 families being used for mapping.

Linkage analysis.

Both RFLP and RAPD markers were mapped relative to resistance loci as two point data using the computer program MAPMAKER (Lander *et al.* 1987). All mapped markers shown were placed with an LOD (log of the odds) score of greater than three, but the distances shown on the genetic map in Figure 3 were calculated manually using the Kosambi mapping function (Koorneef and Stam 1992).

NOTE ADDED IN PROOF

Recent data have shown that RFLP probe m422 was mislabeled and that the probe with which we were provided was in fact m421. Linkage has been detected with probes mapping near to m421 and this would locate *RPP7* on the lower arm of chromosome 1.

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