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RESEARCH ARTICLE

Functional Homologs of the Arabidopsis *RPM1* Disease Resistance Gene in Bean and Pea

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We showed that a bacterial avirulence (*avr*) gene function, *avrPpiA1*, from the pea pathogen *Pseudomonas syringae* pv *pisi*, is recognized by some, but not all, genotypes of Arabidopsis. Thus, an *avr* gene functionally defined on a crop species is also an *avr* gene on Arabidopsis. The activity of *avrPpiA1* on a series of Arabidopsis genotypes is identical to that of the *avrRpm1* gene from *P. s.* pv *maculicola* previously defined using Arabidopsis. The two *avr* genes are homologous and encode nearly identical predicted products. Moreover, this conserved *avr* function is also recognized by some bean and pea cultivars in what has been shown to be a gene-for-gene manner. We further demonstrated that the Arabidopsis disease resistance locus, *RPM1*, conditioning resistance to *avrRpm1*, also conditions resistance to bacterial strains carrying *avrPpiA1*. Therefore, bean, pea, and conceivably other crop species contain functional and potentially molecular homologs of *RPM1*.

INTRODUCTION

Plant disease resistance reactions are often genetically controlled by the simultaneous expression of dominant pathogen functions (avirulence, or *avr*, genes) and corresponding plant loci (resistance, or *R*, genes). This complementarity is the basis of Flor's "gene-for-gene hypothesis" (Flor, 1955, 1971; Ellingboe, 1981, 1982, 1984; Keen, 1982, 1990; Gabriel, 1989), which describes genetically the interactions of plants with bacterial, fungal, and viral pathogens. In particular, the coevolved interactions of biotrophic fungi and bacteria with their respective host species have led to the familiar concept of pathogen race–plant cultivar specificity (Crute, 1985; Pryor, 1987; Clarke et al., 1990; Frank, 1992). However, it is also apparent that the genetic paradigms of Flor's hypothesis may have universal applicability because gene-for-gene recognition can govern host range restriction at the plant species level for both bacterial and fungal pathogens (Keen and Staskawicz, 1988; Whalen et al., 1988, 1991; Kobayashi and Keen, 1989; Tosa, 1989; Keen, 1990; Keen and Buzzell, 1990; Valent et al., 1991; Liu and Rimmer, 1992; Swarup et al., 1992). These findings have led to a speculative, integrated model of how plant defense mechanisms may have evolved to recognize potential pathogens (Heath, 1991) and have blurred the traditional definitions of "host" and "nonhost" plant–pathogen interactions.

A wealth of genetic evidence supports the gene-for-gene nature of recognition events that lead to the resistant phenotype. Nevertheless, the mode of action of either *avr* or *R* gene products remains enigmatic. Several bacterial *avr* genes have been cloned and analyzed, yet no detailed understanding of either their normal function or their ability to trigger the plant's resistance mechanism has emerged (Keen and Staskawicz, 1988; Keen, 1990; DeWit, 1992). More critically, no *R* gene product has been isolated to date. The isolation of *R* genes, and an understanding of their structure and function, may allow a mechanistic clarification of gene-for-gene recognition. The molecular characterization of *R* genes is also necessary if we are to understand how resistance specificities are evolutionarily deployed within and between various plant species, and how that deployment may be engineered for more durable disease resistance.

The molecular genetic advantages of Arabidopsis (Rédei, 1975; Meyerowitz, 1987, 1989) have been recently exploited to investigate the genetic control of plant–pathogen interactions (Susnova and Poljak, 1975; Melcher, 1989; Koch and Slusarenko, 1990a, 1990b; Li and Simon, 1990; Simpson and Johnson, 1990; Ausubel et al., 1991; Bent et al., 1991; Dangl et al., 1991, 1992; Daniels et al., 1991; Davis et al., 1991; Debener et al., 1991; Dong et al., 1991; Simons et al., 1991; Tsuji et al., 1991; Whalen et al., 1991; Uknes et al., 1992; reviewed by Dangl, 1992b). Two approaches have been taken. In the first, natural infections of Arabidopsis were characterized, whereas in the second, pathogens of related cruciferous species were test inoculated into leaves of various Arabidopsis

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genotypes. The demonstration of gene-for-gene specificity with the latter approach (Debener et al., 1991) represents another example of specific control of pathogen recognition across traditionally defined host plant species borders. Based on this result, we wondered whether Arabidopsis harbored resistance genes capable of recognizing *avr* genes previously defined in crop plant species.

In this study, we present evidence that *avr* genes from two divergent pathovar groups of *Pseudomonas syringae* are recognized by Arabidopsis, that these two *avr* genes are nearly identical, and that resistance to bacterial isolates harboring them is determined by the previously described *RPM1* locus of Arabidopsis. These data clearly show that Arabidopsis contains functional, and potentially molecular, homologs of genes active as resistance genes in pea, bean, and soybean.

RESULTS

Genotype-Dependent Recognition of a *P. s.* pv *pisi avr* Gene by Arabidopsis

We asked first whether *avr* gene functions defined in other plant species could detect resistance specificities in Arabidopsis. Cosmid clones each carrying one of four *avr* genes were conjugated into a *P. s.* pv *maculicola* isolate, m4, previously shown to be virulent on a large array of Arabidopsis genotypes (Debener et al., 1991; see Methods). The *avr* genes used were *avrPpiA1* or *avrPpiA2* from *P. s. pisi* races 2 and 3, respectively (Taylor et al., 1989; Vivian et al., 1989; Bavage et al., 1991), and *avrPph3* or *avrPph2* from *P. s.* pv *phaseolicola* races 3 and 4, respectively (Hitchin et al, 1989; Jenner et al., 1991). The bacterial strains and plasmids described in this paper are listed in Methods.

Figure 1A shows the result of inoculating a transconjugant carrying the *avrPpiA1* gene into leaves of Arabidopsis genotype Col-0. Using high-titer bacterial inoculum (above 10^7 [colony-forming units] cfu/mL), the presence of the *avrPpiA1* gene in the *P. s. maculicola* isolate m4 background triggers a rapid tissue collapse indicative of a hypersensitive resistance response (HR) (Klement, 1982). The *P. s. maculicola* isolate m2, shown previously to harbor the *avrRpm1* gene (Debener et al.,1991) (referred to hereafter as *avrPmaA1*), served as a positive control in this experiment. This experiment was repeated with an expanded panel of test Arabidopsis genotypes, and representative results are presented in Figure 1B. Mt-0 and Nd-0 are among the five susceptible genotypes incapable of generating an HR in response to the presence of the *avrPpiA1* gene in m4, whereas Col-0 is one of nine resistant genotypes that do respond. These results are summarized in Table 1. An unexpected and noteworthy aspect of these results is that the distribution of Arabidopsis genotypes resistant and susceptible to *avrPpiA1* is identical to that of the previously described *avrPmaA1* gene from *P. s. maculicola*. We confirmed these phenotypic observations by measuring bacterial growth in leaves following inoculation of selected Arabidopsis genotypes at low titer (10^5 cfu/mL). The results,

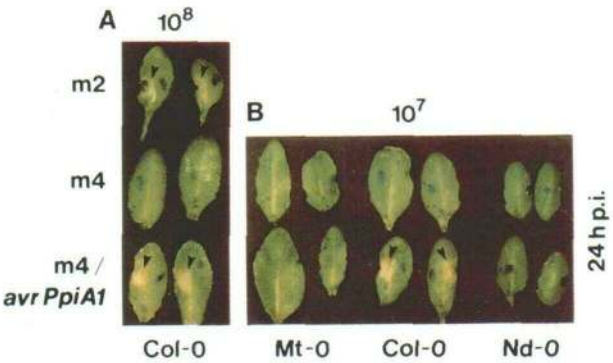

Figure 1. The *avrPpiA1* Gene from *P. s. pisi* Also Functions as an *avr* Gene on Arabidopsis.

(A) Recognition of *avrPpiA1* by genotype Col-0 after inoculation at 10^8 cfu/mL.

(B) Arabidopsis genotype-dependent recognition of *avrPpiA1* after inoculation at 10^7 cfu/mL.

Leaves were marked with either black or blue marking pen, and bacteria were infiltrated into a small area across from each spot. As shown here, and discussed in detail by Debener et al. (1991), the virulent phenotype of *P. s. maculicola* isolate m4 is not visible at this early time point. **(A)** and **(B)** are from different experiments, which were each performed twice with each bacterial concentration. Six to 12 leaves from two or three plants were inoculated with each density of bacteria in each experiment. Representative necrotic phenotypes (arrowheads) presented here were observed in each leaf. p.i., postinoculation.

Table 1. Avirulence Specificities of *avrPpiA1* and *avrPmaA1* Are Identical on Arabidopsis

	Arabidopsis Genotypes												
	Bl-1	Col-0	Cvi-0	Fe-1	Hi-0	La-er	Mt-0	Nd-0	Oy-0	Per-0	Pi-0	Stw-0	Ta-0
P. s. maculicola:													
m4	C	C	C	C	C	C	C	C	C	C	C	C	C
m4/*avrPmaA1*	I	I	C	C	I	I	C	C	I	I	I	I	I
m4/*avrPpiA1*	I	I	C	C	I	I	C	C	I	I	I	I	I

I indicates incompatible interaction; the HR occurs between 10 and 20 hr, depending on initial inoculum density. C designates the compatible interaction that is marked by water soaking and/or chlorosis: it was assayed at bacterial titer of 10^7 and 10^8 cfu/mL over a 3-day time course.

shown in Figure 2, indicate clearly that the *avrPpiA1* gene function suppresses bacterial growth of *P. s. maculicola* isolate m4 only in leaves of the HR^+ Col-0 genotype. These data showed that the *avrPpiA1* gene functions as an *avr* gene on Arabidopsis and detects resistance specificity analogous to that of the *avrPmaA1* gene.

The other three tested *avr* genes from *P. s. pisi* and *P. s. phaseolicola* did not generate plant genotype-dependent resistance reactions on Arabidopsis and will not be considered further (data not shown).

The *P. s. pisi avrPpiA1* and *P. s. maculicola avrPmaA1* Genes Are Nearly Identical

The data described above prompted us to ask whether the *avrPpiA1* and *avrPmaA1* genes are structurally related. We had delimited *avrPmaA1* activity to a 2.5-kb fragment (C. Ritter, unpublished data) and present further definition via transposon mutagenesis as given below. DNA gel blots using the 2.5-kb insert of pCR104 (see Methods) as probe against several cloned *avr* genes showed clear hybridization to only the *avrPpiA1* gene (C. Ritter, data not shown). We then surveyed the distribution of *avrPmaA1* in other *P. s. maculicola* isolates, and to other *Pseudomonas* and *Xanthomonas* strains using a small 0.7-kb probe containing essentially only the *avrPmaA1* coding region. Other than the strain from which *avrPmaA1* was isolated (*P. s.* pv *maculicola* m2), only isolates 791 (m6 in Debener et al., 1991), 90, 755, and 10,832 carried the same 7.5-kb EcoRI fragment that strongly hybridizes to *avrPmaA1*, as shown in Figure 3. When present, the *avrPmaA1* gene is borne on a plasmid of approximately 35 kb in *P. s. maculicola* m2 in the tested *P. s. maculicola* isolates (C. Ritter, unpublished data). No hybridization was observed to genomic DNA from either one isolate each of *P. syringae* pvs *tabaci*, *glycinea*, *aptata*, *phaseolicola*, *angulata*, or *mellea*, or to a race 1 isolate of pv *pisi*. There was also no hybridization to any of nine *P. cichorii* strains and none to any of 18 *X. campestris* isolates of various pathovar designations (C. Ritter, data not shown). Similarly, a probe of 4.1 kb containing the *avrPpiA1* gene detected strong homology to strains 65A, 1819A, and 1853A of *P. s. maculicola*, as well as to strain 802 of the normally saprophytic *P. viridiflava* (Mur, 1991). Because this larger probe contains a great deal of nucleotide sequence flanking *avrPpiA1*, these data are inconclusive.

The *avrPpiA1* probe was also used to clone the hybridizing fragment from isolate 791 in plasmid pAV500. Functional attributes of this homolog are described below. These hybridization data showed that the homologous *avrPpiA1* and *avrPmaA1* genes occur in several isolates of different *P. syringae* pathovars, but they are not widely distributed, at least within

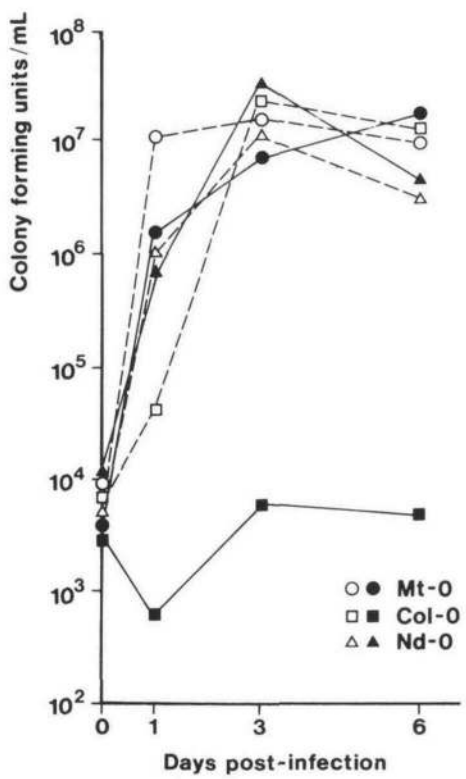

Figure 2. Presence of the *avrPpiA1* Gene Limits Growth of a Normally Virulent *P. s. maculicola* Strain in a Plant Genotype-Dependent Manner.

P. s. maculicola isolate m4 (open symbols) or m4 transconjugants carrying the *avrPpiA1* gene on pAV200 (filled symbols) were inoculated into leaves of three different Arabidopsis genotypes at 10^5 cfu/mL. Bacterial growth in leaves was monitored over a 6-day period as described previously (Dangl et al., 1991; Debener et al., 1991). Data presented are from one of two independent experiments.

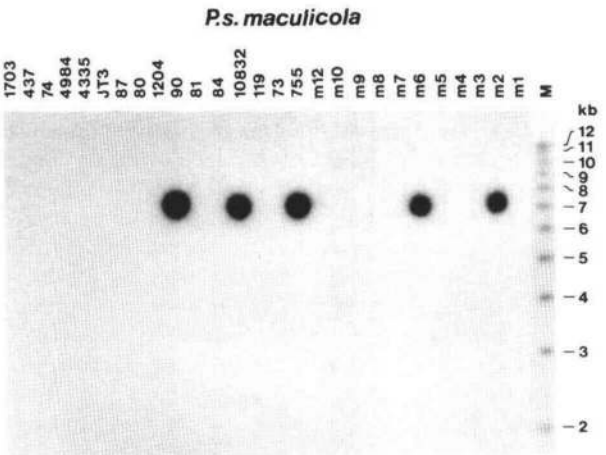

Figure 3. Several *P. s. maculicola* Isolates Carry Sequences Homologous to the *avrPmaA1* Gene.

Total genomic DNA (2 μg) was digested with EcoRI, separated on an agarose gel, blotted, and probed with a 0.7-kb PstI-EcoRI fragment from pCR102 carrying the *avrPmaA1* gene (Figure 4).

the limited scope of our survey. The occurrence of these two *avr* genes in different nominal pathovar groups underscores the tenuous nature of pathovar designation and nomenclature.

Transposon mutagenesis using Tn*3*spice (for *avrPmaA1*) and both Tn*5* and Tn*3*HoKmgus (for *avrPpiA1*) was performed for further localization of both *avr* genes (see Methods). Figure 4 presents comparative restriction map and insertion inactivation data for each *avr* gene. Both *avr* activities map to analogous, small regions of the respective subclones. An approximately 1-kb region of pCR102 carries *avrPmaA1* activity as defined by insertion mutagenesis and functional analysis on Arabidopsis, whereas a 1.5-kb region of pAV200 contained the *avrPpiA1* gene activity as assayed on pea (see below). Inactivation of *avr* function in both cases results in loss of recognition by Col-0 of *P. s. maculicola* m4 transconjugants carrying transposon insertions, as shown in Figure 5. The active region of pAV200 was subcloned and sequenced. Primers derived from the *avrPpiA1* sequence were then used to sequence the *avrPmaA1* gene.

A comparison of the DNA sequences of both *avr* genes is presented in Figure 6A. The two genes share 97% nucleotide identity. Only one extended open reading frame exists in each sequence, and the conceptual translations for both genes are compared in Figure 6B. The deduced amino acid sequences are also 97% identical. The *avrPmaA1* homolog cloned from *P. s. maculicola* isolate 791 is identical to *avrPmaA1* (M. J. Gibbon, unpublished results). The predicted protein product of molecular mass 28 kD is strikingly hydrophilic, and it shares no significant similarity to any sequence in the GenBank, EMBL, or Swiss-Prot data bases, including the many known *avr* gene products. There is a weak homology with the *cis*-regulatory, *hrp* box (Jenner et al., 1991) found upstream of several other *avr* gene sequences at about 55 bp upstream of the ATG translation initiation. Thus, as with all *avr* genes cloned and analyzed to date, the function of the protein encoded by these homologous *avr* genes remains unknown.

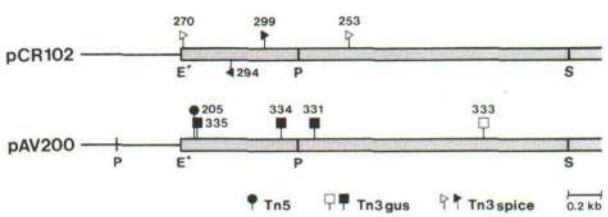

Figure 4. Localization of *avrPpiA1* and *avrPmaA1* by Transposon Mutagenesis.

pCR102 (Table 3) carrying *avrPmaA1* was mutagenized with Tn*3*spice. Insertions were mapped to determine the site and orientation of transcription (left to right above the line, right to left below). pAV200 (Table 3) was mutagenized with either Tn*5* or Tn*3*HoKmgus. For both clones, solid symbols represent HR insertions, and open symbols show HR$^+$ insertions. Numbers for each insertion refer to plasmid designations (Table 3). Restriction sites shown are E, EcoRI (asterisk denotes site donated by the vector polylinker); P, PstI; S, SstI.

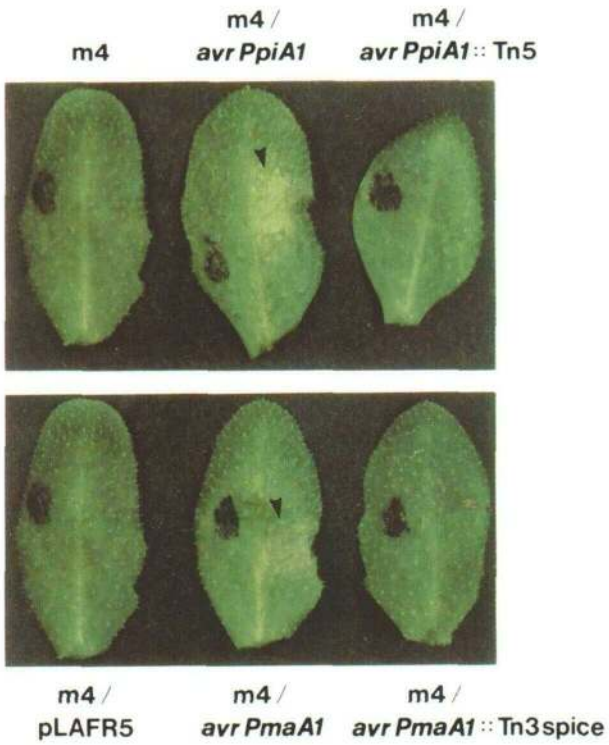

Figure 5. Transposon Insertions in *avrPpiA1* and *avrPmaA1* Abolish the HR on Arabidopsis Genotype Col-0.

P. s. maculicola isolate m4 or various transconjugants were inoculated into Col-0 leaves at 10^8 cfu/mL opposite marking pen spots. The HR was observed at 6 hr postinoculation in this experiment. Clones used were either pCR102 or pCR294 for *avrPmaA1* and either pAV200 or pAV205 for *avrPpiA1* (Figure 4). As shown in Figure 1 and Debener et al. (1991), the virulent phenotype of *P. s. maculicola* isolate m4 is not visible at this early time point. Leaves shown are representative of two independent experiments of two or three plants (six to 12 leaves) per experiment. Arrowheads mark sites of necrosis.

The *P. s. pisi avrPpiA1* and *P. s. maculicola avrPmaA1* Gene Functions Are Recognized Identically by at Least Three Plant Species

Fillingham et al. (1992) recently showed that presence of the *avrPpiA1* gene in an otherwise virulent *P. s. phaseolicola* race 6 isolate led to the HR on some bean cultivars. In particular, they showed that cultivars Canadian Wonder and Seafarer were able to recognize the *avrPpiA1*-containing strain. The HR on pods of cultivar Seafarer was phenotypically different from that on Canadian Wonder in that it was slower to develop. Genetic segregation analysis indicated the presence of single loci in the respective cultivars conferring each type of resistance phenotype (Fillingham et al., 1992). These resistance specificities are unlike any in bean triggered by characterized races of *P. s. phaseolicola*. Based on both the sequence homology of *avrPpiA1* and *avrPmaA1* and their functional homology on

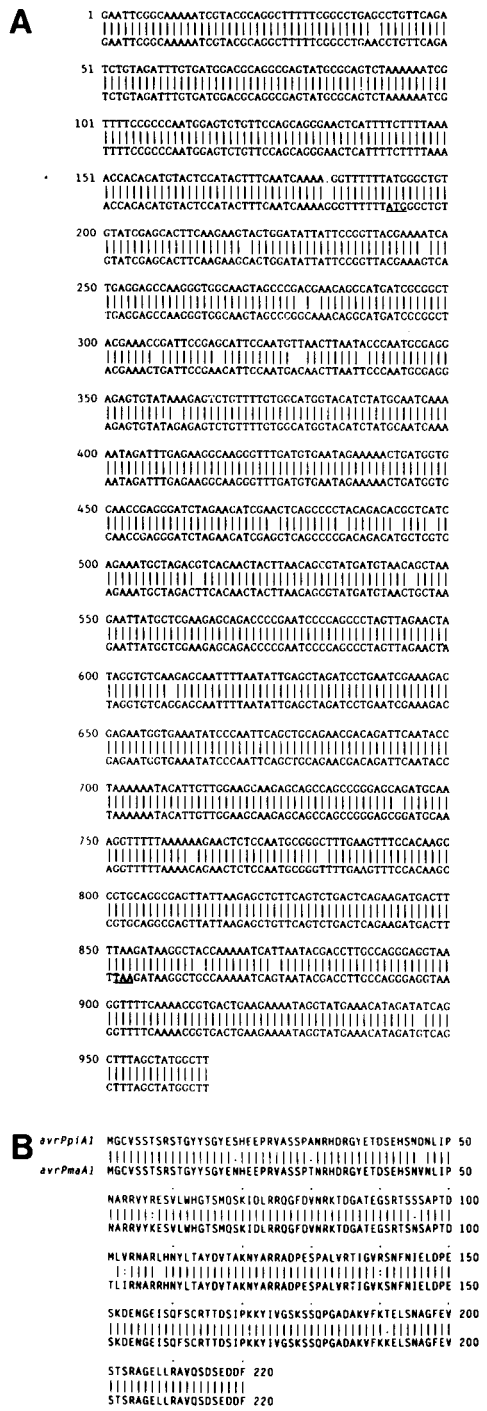

Figure 6. The *avrPpiA1* and *avrPmaA1* Genes Are Nearly Identical.

(A) Nucleotide sequences of *avrPmaA1* (upper line) and *avrPpiA1* (lower line). Translation initiation and termination codons of the putative proteins are underlined.

(B) Predicted amino acid sequences of both *avrPpiA1* (upper line) and *avrPmaA1* (lower line) gene products. Dots show intermediate nonconservative exchanges; colons indicate conservative changes. EMBL accession numbers are X67807 for *avrPpiA1* and X67808 for *avrPmaA1*.

Arabidopsis, we predicted that *avrPmaA1* would also trigger a cultivar-specific HR when tested on these two differential bean cultivars. We further predicted that *avrPmaA1* would also be recognized by pea cultivars, such as Martus, that carry the *R2* resistance locus, conditioning resistance to strains containing *avrPpiA1* (*P. s. pisi* race 2) (Taylor et al., 1989; Vivian et al., 1989). Table 2 summarizes functional tests on all three plant species.

Both *avr* genes and the *avrPmaA1* homolog carried on pAV500 generated identical reactions on the appropriate test cultivars of bean and pea, in addition to Arabidopsis as described above, and soybean (N. T. Keen, personal communication). Although a limited number of pea and bean cultivars were tested, they represent critical tests of our predictions. Moreover, transposon insertions into the open reading frame conserved among the three genes abolished recognition by resistant genotypes of all three plant species. Thus, these *avr* genes are functionally equivalent in their ability to convert appropriate virulent *P. syringae* isolates to avirulence in a plant genotype–dependent manner on at least four species.

The Arabidopsis *RPM1* Resistance Locus Also Encodes Resistance to *avrPpiA1*

That *avrPpiA1* and *avrPmaA1* are functional and molecular homologs strongly suggests that the same plant resistance locus recognizes their signal. To prove this point, we tested *avrPpiA1* function on families of F_3 Arabidopsis plants derived from selfed F_2 individuals from the cross Col-0 × Nd-0. These plants were identified previously and used in restriction fragment length polymorphism mapping of the *RPM1* resistance locus, conditioning resistance to *P. s. maculicola* strains carrying *avrPmaA1* (Debener et al., 1991). The genotypes of 30 F_2 individuals had been previously deduced as either homozygous resistant (*RPM1*/*RPM1*, $n = 15$) or homozygous susceptible (*rpm1*/*rpm1*, $n = 15$) based on the HR phenotype following infiltration of F_3 progeny with isolate m2, which harbors *avrPmaA1*. We infiltrated nine individuals from each F_3 family with *P. s. maculicola* isolate m4 carrying the *avrPpiA1* gene and scored for the development of the HR 10 to 15 hr later. Each member of all *RPM1*/*RPM1* families developed the HR, and no individuals from *rpm1*/*rpm1* families developed the HR (data not shown). The likelihood of this result occurring by chance is 10^{-9} (Allard, 1956). Although it is still theoretically possible that a gene very tightly linked to *RPM1* is recognizing the *avrPpiA1* signal, we consider this unlikely given the high sequence homology between the two predicted *avr* gene products.

DISCUSSION

Two significant conclusions emerge from the data presented here. The first concerns distribution of *avr* functions among

Table 2. Functional Homology of *avrPmaA1* and *avrPpiA1* on Arabidopsis, Bean, and Pea

Plant Genotypes	Arabidopsis		Bean		Pea	
	Col-0	Nd-0	Canadian Wonder	Seafarer	Kelvedon Wonder	Martus
Bacteria						
P. s. maculicola m2	I	C	I	I	NT	NT
P. s. maculicola m6 (791)	I	C	I	I	I	I
P. s. maculicola m4 (4326)	C	C	I	I	I	I
P. s. phaseolicola 1448A	Null	Null	C	C		
P. s. pisi PT10	NT	NT	I	I	C	C
Transconjugants in						
P. s. maculicola m4/			*P. s. phaseolicola* 1448A/			*P. s. pisi* PT10/
avrPmaA1(pCR102)	I	C	I	I*	C	I
avrPpiA1(pAV200)	I	C	I	I*	C	I
avrPmaA2(pAV500)	I	C	I	I*	C	I
avrPmaA1:: Tn3spice (pCR294)	C	C	C	C	C	C
avrPpiA1:: Tn*5* (pAV205)	C	C	C	C	C	C
Vector (pLAFR3 or 5)	C	C	C	C	C	C

I indicates incompatible interaction, HR observed; I*, delayed HR as described in Fillingham et al. (1992); C, compatible interaction marked by water soaking and/or chlorosis; Null, no visible symptom; NT, not tested. See Methods for details of assays on the three plant species.

P. syringae pathovars that can be recognized by a wide variety of plant species. The second is that a well-characterized resistance function from a model plant has functional homologs in several crop species. The cloning and structural analysis of the Arabidopsis *RPM1* locus may offer clues into the distribution of this resistance function across a wide variety of species.

That the *avrPpiA1* gene is recognized by Arabidopsis is not extremely surprising because the phenomenon of genotype-specific recognition of *avr* functions by nominally nonhost species has been described. Previously, Kobayashi et al. (1989) identified four *avr* functions from *P. s. glycinea* and *P. s.* pv *tomato* that detected resistance specificities in soybean. This observation allowed subsequent genetic identification of four corresponding soybean loci (Keen and Buzzell, 1991). Whalen et al. (1988) isolated an *avr* gene from *X. c.* pv *vesicatoria* (a tomato and pepper pathogen) that detected a novel resistance gene function in bean and was recognized by five additional plant species. These studies stimulated the recent identification of two new *avr* genes, defined on Arabidopsis, from *P. s. tomato* and *P. s. maculicola* (Debener et al., 1991; Dong et al., 1991; Whalen et al., 1991). The *avrRpt2* gene, defined on Arabidopsis, also triggered the HR when tested on soybean (Whalen et al., 1991), turnip, and radish (Dong et al., 1991).

Illusory delineation of the borders between "host" and "nonhost" plant–pathogen interactions is not confined to bacterial pathosystems. Two recent examples also show that genetic definition of host range–limiting functions can uncover cryptic gene-for-gene interactions in fungal pathosystems (Tosa, 1989; Valent et al., 1990). It has often been suggested that many, but certainly not all, nonhost interactions are governed by a nonepistatic series of gene-for-gene interactions (reviewed by Keen and Staskawicz, 1988). This would require that a pathogen simultaneously lose one, or more, *avr* functions to broaden its host plant range through mutation.

Alternatively, specific recognition by a nominal nonhost species may, at least in some cases, not be the factor limiting host plant range. Swarup et al. (1992) showed that a pathogenicity gene (*pthA*) from *X. citri* also functions as an *avr* gene in *X. c.* pv *malvacearum* and triggers the HR on soybean when present in *X. c.* pv *glycinea.* However, marker exchange deletion of the *pthA* gene did not render *X. citri* pathogenic on either cotton or soybean. Thus, recognition of a given *avr* function does not necessarily imply that its action alone is responsible for observed limitation of host range. An interesting model to place current observations into an evolutionary context was recently outlined by Heath (1991).

The recognition of *avr* functions by plant species other than the nominal host is still an exception rather than a rule. Moreover, in the cases cited above, experimental strategies were adopted to identify *avr* functions that were recognized by nominally nonhost plant species. Data presented by Fillingham et al. (1992) and in this work show that an *avr* gene defined via its function within a particular plant species is also recognized as an *avr* gene by other plant species. Can any generalities be found to suggest which *avr* genes will also act as host range–restricting functions?

The *avrPpiA1* and *avrPmaA1* genes are distributed among several *P. syringae* pathovars, and at least the latter is plasmid borne. It is not clear whether this plasmid is self-transmissible

or if it confers a selective advantage on strains carrying it. Many examples of plasmid-borne *avr* genes exist (Staskawicz et al., 1987; Swanson et al., 1988; Tamaki et al., 1988; Bonas et al., 1989; Kobayashi et al., 1990; Bavage et al., 1991). In only one case, however, was it shown that the plasmid conferred an easily understood selective advantage (copper resistance) (Swanson et al., 1988). There is also no strict correlation between plasmid localization and *avr* gene recognition by nonhost plant species. This is evidenced by the fact that the *avrRxv* gene (Whalen et al., 1988) is not plasmid borne but does encode a function recognized by several plant species, and by our observation that the *avrPpiA3* gene, which is plasmid borne, is not recognized by at least Arabidopsis and bean (this study; Fillingham et al., 1992). This conundrum will only be resolved through a detailed understanding of the normal function of both the *avr* gene products and the plant molecules with which they interact.

Our most important finding is that the Arabidopsis *RPM1* locus conditions resistance to the *avrPpiA1* gene, as well as to the *avrPmaA1* gene. This was predictable based on the high homology between the two *avr* gene products. Nevertheless, it was important to demonstrate that either the same gene at the *RPM1* locus or two very closely linked genes mediate recognition of both *avr* gene functions. If two closely linked *R* gene specificities do mediate recognition of the two *avr* gene products, then they are at most 1.8 map units apart. This conclusion is based on no observed recombinants for resistance to the two *avr* gene functions among 30 progeny from homozygous F_2 individuals tested (see Debener et al., 1991). If, in future analyses of other families defined as homozygous at *RPM1* a recombinant is found that, for example, is *RPM1/RPM1* when tested with *avrPmaA1* but segregates for resistance to *avrPpiA1*, then two closely linked *R* genes are present. Tightly clustered resistance specificities encoding fungal resistance are nearly the rule (Saxena and Hooker, 1968; Shepherd and Mayo, 1972; Hulbert and Michelmore, 1985; Pryor, 1987; Bennetzen et al., 1991; Jorgenson, 1991; Dangl, 1992a) but are rather the exception for bacterial resistance specificities (Yoshimura et al., 1983). Finding a recombinant would also suggest that the amino acid substitutions between the two *avr* gene products are of functional relevance.

The *RPM1* gene is currently being cloned and has been localized to less than 200 kb of Arabidopsis DNA (T. Debener, H. Liedgens, M. Gerwin, and J. L. Dangl, unpublished data). Isolation of *RPM1* will allow direct testing of its functional efficacy in bean, pea, and soybean, and will hopefully provide probes to isolate the corresponding genes from those crop species. Due to the recognition of *avrPpiA1* by Arabidopsis, it will also be possible to test bean and pea DNA clones in Arabidopsis for *R* gene activity, overcoming the still tedious nature of transformation in those species. Finally, there are at least five *R* gene specificities in pea, and also in bean, postulated to recognize the known races of *P. s. pisi* and *P. s. phaseolicola* (Taylor et al., 1989; Jenner et al., 1991). Through the functional identity of *avrPpiA1* and *avrPmaA1*, and their recognition mediated by a known Arabidopsis locus, it may be possible to isolate all the corresponding resistance genes from bean and pea via homology, assuming, of course, that a functionally relevant *R* gene domain is conserved. Availability of these would greatly further our understanding of specific plant–pathogen recognition.

METHODS

Maintenance of Bacteria

Pseudomonas syringae strains, as given in Table 3, were grown on King's B media (King et al., 1954) shaken at 25 to 28°C. *Escherichia coli* strains (Table 3) were grown in Luria-Bertani (LB) broth or on LB agar plates at 37°C (Maniatis et al., 1989). For *Pseudomonas* strains, antibiotics were used at the following concentrations (mg/L): rifampicin, 50; tetracycline, 10; nalidixic acid, 50; spectinomycin, 20 to 100. For *E. coli* strains (Table 3), antibiotics were used as follows (mg/L): ampicillin, 100; tetracycline, 5 or 15; nalidixic acid, 10 to 50 (see Table 3); kanamycin, 30 to 50; spectinomycin, 10; streptomycin, 50.

Plasmid Subcloning and DNA Sequencing

Plasmids are listed in Table 3. Those containing *avrPmaA1* were derived from cosmid K48 (Debener et al., 1991). All molecular manipulations were done via standard procedures (Ausubel et al., 1987; Maniatis et al., 1989). Sequencing of double-stranded DNA was performed according to Sanger et al. (1977), as modified by Tabor and Richardson (1987), using the Sequenase version 2.0 kit (U.S. Biochemical Corp.).

Bacterial Genomic DNA Preparation and DNA Gel Blotting

Genomic DNA was prepared according to Ausubel et al. (1987): digests were prepared, and gel electrophoresis, blotting, probe preparation, and hybridization were all performed according to standard procedures. High-stringency washing was with 0.1 × SSC (1 × SSC is 0.15 M NaCl, 0.015 M sodium citrate) containing 0.4% SDS at 65°C for 2 × 20 min.

Triparental Mating

Conjugations from *E. coli* DH5α or HB101 were performed via a modification (Debener et al., 1991) of standard protocols using pRK2013 as a helper plasmid (Ditta et al., 1982). Briefly, 2-mL cultures, containing appropriate antibiotics, of donor *E. coli*, recipient *Pseudomonas*, and *E. coli* carrying a helper plasmid were grown overnight and then centrifuged at 4500 rpm for 5 min and resuspended in 1 mL of 10 mM $MgCl_2$. The three bacterial strains were mixed in equal volume and a 100-μL spot was air dried onto a King's B media plate containing no antibiotics. After incubation overnight at 28°C, bacteria were streaked onto media selective for the desired transconjugant. Resulting colonies were colony purified, and cosmids were analyzed by the method of Kado and Liu (1980). Alternatively, 1 mL of a 2:1:1 volume ratio of recipient, donor, and helper plasmid carrying strain from cultures grown overnight was briefly centrifuged, resuspended in 100 μL of sterile water, and spotted for conjugation overnight at 30°C. Plasmids could also be "back-conjugated" into *E. coli* for facile DNA analysis.

Table 3. Bacterial Strains and Plasmids Used in This Study

Designations	Genotypes, Relevant Features[a]	Reference
E. coli		
HB101	F⁻, *recA*, *rpsL20*, Sm^r	Boyer and Roulland-Dussoix (1969)
DH5α	Nal^r (10 μg/mL), F⁻, *recA*, *80dlacZ*, *M15*	Bethesda Research Laboratories
C2110	Nal^r (50 μg/mL), *polA*	Stachel et al. (1985)
P. syringae pv		
maculicola isolates		
m2	Rif^r	Debener et al. (1991)
m4 (4326)	Rif^r	Debener et al. (1991)
m6 (HRI 791)	Rif^r	Debener et al. (1991)
(All strains in Figure 3)	Rif^r	Debener et al. (1991); or gift of B. J. Staskawicz
pisi isolates		
PT10	Rif^r race 4-like derivative of PT2	P.J. Moulton, Bristol Polytechnic
phaseolicola isolates		
1448AR	Rif^r derivative of race 6 1448A	Fillingham et al. (1992)
Plasmids		
pSShe	*tnpA*⁺, pACYC184 replicon, Cm^r	Stachel et al. (1985)
pTn*3*spice	*inaZ*⁺, Ap^r, Sp^r, Sm^r	Lindgren et al. (1989)
pTn*3*HoKmgus	*tnpA*⁺, Km^r, Ap^r, promoterless β-glucuronidase gene	Bonas et al. (1989)
pRK2013	Km^r, Tra⁺, Mob⁺, ColE1 replicon	Figurski and Helinski (1979)
pLAFR3 or pLAFR5	Tra⁺, Mob⁺, RK2 replicon, Tc^r	Staskawicz et al. (1987); Keen et al. (1988)
pSPT19	Ap^r, pUC19 derivative	Boehringer-Mannheim
pCR102	A 6-kb EcoRI fragment from pCR100 cloned into pLAFR5; carries *avrPmaA1*	C. Ritter, unpublished data
pCR104	Derived from pCR102 in two steps: A 2.5-kb EcoRI-SstI fragment from pCR102 was subcloned in pSTP19. This donates a BamHI site. The insert was excised as an EcoRI-BamHI fragment and cloned into pLAFR5.	C. Ritter, unpublished data
pCR294, 299	Tn*3*spice insertions into pCR102	This study
pAV270	A 4.2-kb EcoRI fragment; contains *avrPpiA1*	Vivian et al. (1989)
pAV205	Tn*5* insertions into pAV200	Atherton (1987)
pAV331,333,334,335	Tn*3*Gus insertions into pAV200	This study

[a] Sm, streptomycin; Rif, rifampicin; Cm, chloramphenicol; Ap, ampicillin; Sp, spectinomycin; Km, kanamycin; ^{r}, resistant.

Transposon Mutagenesis

Mutagenesis with Tn*3*spice was modified from the original reference (Lindgren et al., 1989) and subsequent reference (Bonas et al., 1989) as follows. The target plasmid was transformed into HB101 containing pTn*3*spice (or Tn*3*HoKmgus) and pSShe, and several independent transformants were selected on the appropriate antibiotics. A pool of 10 transformants was conjugated en masse into *E. coli* C2110. Fresh strains were grown as plate stocks, and 3-mL cultures of either helper (HB101 with pRK2013), recipient (C2110), or the pool of 10 transformants were grown in LB broth without antibiotics at 37°C for 90 to 120 min, to an optical density (OD) representing 10^8 colony-forming units (cfu) per mL. Spots (100 μL) of each strain were mixed on an LB plate (no antibiotics) and incubated overnight at 37°C. The conjugation mix was collected and resuspended in 1.2 mL of LB broth; 200 μL was plated onto each of six LB plates containing nalidixic acid (50 μg/mL), tetracycline, and kanamycin and grown overnight at 37°C. All colonies were scraped off each plate independently and resuspended in LB broth. Cells were pelleted and miniprep DNA prepared essentially as described by Maniatis et al. (1989), except that an LiCl step was included to remove RNA. The final DNA pellet was resuspended in 10 to 30 μL of sterile water; 5 μL was used to transform competent DH5α, and transformants containing transposon insertions into the pLAFR5 plasmid were selected on nalidixic acid (10 μg/mL), tetracycline (5 μg/mL), and spectinomycin (10 μg/mL). Mutagenesis with Tn*5* was as described by Turner et al. (1985).

Care of Plants

Arabidopsis thaliana plants were maintained as described by Dangl et al. (1991) and Debener et al. (1991). Conditions for pea (Vivian et al., 1989) and bean (Harper et al., 1987) have also been described previously.

Inoculations of Plants

Arabidopsis leaves were inoculated with bacteria in exactly the manner described by Debener et al. (1991). Bacteria were prepared as described by Debener et al. (1991), except that overnight cultures of OD_{600} = 1.0 to 1.5 were diluted to OD_{600} = 0.1 and allowed to grow for 1 to 2 hr before washing and adjustment to the desired density for inoculation. Alternatively, overnight cultures were washed and adjusted to the desired OD_{600}. An OD_{600} of 0.2 was taken as roughly 10^8 cfu/mL. Inoculation of bean pods (Fillingham et al., 1992) and pea stems (Malik et al., 1987) has been previously detailed.

Statistical Considerations

The probability discussed in the text was calculated as $P = 2[1 - (1/4^n)(1/4^n)]$, where n is the number of families in each homozygous class (after Allard, 1956; Michelmore et al., 1991).

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