Soybean Resistance Genes Specific for Different *Pseudomonas syringae* Avirulence Genes are Allelic, or Closely Linked, at the *RPG1* Locus

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ABSTRACT

RPG1 and *RPM1* are disease resistance genes in soybean and Arabidopsis, respectively, that confer resistance to *Pseudomonas syringae* strains expressing the avirulence gene *avrB*. *RPM1* has recently been demonstrated to have a second specificity, also conferring resistance to *P. syringae* strains expressing *avrRpm1*. Here we show that alleles, or closely linked genes, exist at the *RPG1* locus in soybean that are specific for either *avrB* or *avrRpm1* and thus can distinguish between these two avirulence genes.

RESISTANCE displayed by particular plant cultivars to specific races of a pathogen is often mediated by single dominant resistance genes (R-genes). Typically, these R-genes interact with single dominant "avirulence" (*avr*) genes in the pathogen. Such specific interactions between races of pathogens and cultivars of host plants are the basis of the "gene-for-gene" model of disease resistance developed by H. H. FLOR over 50 years ago (FLOR 1955). This model states that resistance of a plant cultivar to a specific pathogen race is controlled by a single dominant resistance gene, the product of which specifically interacts (directly or indirectly) with the product of a "corresponding" avirulence gene. Thus, for each avirulence gene in the pathogen, there is a corresponding resistance gene in a resistant plant, and resistance is observed only when both genes are present. Often this resistance is associated with a "hypersensitive resistance response" (HR) that is visualized as rapid localized necrosis of plant tissue at the infection site. The HR appears to be an important component of the defense response in many plant species (GOODMAN and NOVACKY 1994).

Recently, the "gene-for-gene" model has been extended beyond race–cultivar interactions to include interactions between plant pathogens and "nonhosts." For example, the tomato pathogen *Pseudomonas syringae* pv. *tomato* (*Pst*) possesses multiple avirulence genes that, when expressed in *P. syringae* pv. *glycinea* (*Psg*), induce an HR in various cultivars of soybean (KOBAYASHI *et al.* 1989). This interaction was shown to be a true gene-for-gene interaction when KEEN and BUZZELL (1991) established that the resistance response in specific soybean cultivars was controlled by single dominant resistance genes corresponding to the individual *Pst* avirulence genes. Thus, soybean cultivars carry resistance genes specific to avirulence genes of both the soybean pathogen *Psg* and the tomato pathogen *Pst*. The inability of *Pst* to cause disease in any soybean cultivar can be explained, at least in part, by the presence of a battery of resistance genes in soybean that correspond to one or more avirulence genes present in all *Pst* strains.

There are now several examples of bacterial *avr* genes detected by multiple plant species (WHALEN *et al.* 1991; DANGL *et al.* 1992; FILLINGHAM *et al.* 1992; RONALD *et al.* 1992; INNES *et al.* 1993; SIMONICH and INNES 1995). These studies suggest that R-genes sharing the same specificities are present in different plant species. This has been demonstrated genetically for interactions involving *avrB* (KEEN and BUZZELL 1991; INNES *et al.* 1993), *avrRpm1* (VIVIAN *et al.* 1989; DEBENER *et al.* 1991; FILLINGHAM *et al.* 1992) and *avrPph3* (JENNER *et al.* 1991; SIMONICH and INNES 1995). It is unclear as to whether this phenomenon represents the conservation of ancestral R-genes through speciation or whether convergent evolution is responsible. If functionally analogous R-genes in different species represent the conservation of ancestral genes during speciation, it seems paradoxical that they should be lost (or change specificity) at a high frequency within a species; however, multiple alleles of differing specificities is a hallmark of R-gene loci (PRYOR and ELLIS 1993). The cloning of R-genes sharing common specificities will help address this question.

Only recently have the first three R-genes specific for bacterial *avr* genes been cloned. These are the *Pto* gene from tomato and *RPS2* and *RPM1* from Arabidopsis (MARTIN *et al.* 1993; BENT *et al.* 1994; MINDRINOS *et al.* 1994; GRANT *et al.* 1995). *Pto* and *RPS2* interact with the *P. syringae* avirulence genes *avrPto* and *avrRpt2*, respectively (RONALD *et al.* 1992; KUNKEL *et al.* 1993). *RPM1* displays a dual specificity, responding to both *avrRpm1* and *avrB* and consequently is also known as *RPS3* (DEBENER *et al.* 1991; INNES *et al.* 1993; BISGROVE *et al.* 1994; GRANT *et al.* 1995). Sequence analysis has revealed that

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TABLE 1

Bacterial strains and plasmids used

Bacterial strain/ plasmid	Description	Reference
Strain		
*Psg*R4	*Pseudomonas syringae* pv. *glycinea* race 4 (rifamycin resistant isolate)	LONG *et al.* (1985)
*Psg*R4(*avrB*)	*Psg*R4 carrying the plasmid pVB01	INNES *et al.* (1993)
*Psg*R4(*avrB*::Ω)	*Psg*R4 carrying the plasmid pVB01::Ω	INNES *et al.* (1993)
*Psg*R4(*avrRpm1*)	*Psg*R4 carrying the plasmid pVSP61/*avrRpm1*	This paper
Plasmid		
pVB01	*avrB* cloned in the vector pVSP61	INNES *et al.* (1993)
pVB01::Ω	*avrB* disrupted by the insertion of an Ω fragment cloned in the vector pVSP61	INNES *et al.* (1993)
pVSP61/*avrrPm1*	*avrRpm1* cloned in the vector pVSP61	BISGROVE *et al.* (1994)

Pto shows homology to known serine-threonine protein kinases, suggestive of a role in signal transduction. In contrast, *RPS2* and *RPM1* display no homology to protein kinases but contain leucine-rich-repeats, a putative leucine zipper and a potential nucleotide binding domain. These motifs are also present in other recently cloned R-genes corresponding to viral and fungal pathogens, but their role in R-gene function is unknown (reviewed by BRIGGS 1995; DANGL 1995; INNES 1995; STASKAWICZ *et al.* 1995). Neither is it known whether any of these R-gene products interact directly with pathogen-derived elicitors.

We have been analyzing the R-genes *RPM1* and *RPG1* from Arabidopsis and soybean, respectively. Both genes confer resistance to *P. syringae* strains expressing the avirulence gene *avrB* (MUKHERJEE *et al.* 1966; KEEN and BUZZEL 1991; INNES *et al.* 1993). However, it was not known whether *RPG1,* like *RPM1,* also confers resistance to *Psg* strains expressing *avrRpm1*. Here we show that in most soybean cultivars, *RPG1* is specific only to *avrB*. However an R-gene specific to *avrRpm1* is present in some cultivars, and this gene is closely linked, or allelic, to *RPG1*. We also demonstrate that in a soybean cultivar responsive to both *avr* genes, both resistance specificities are determined either by an allele of *RPG1* or by *RPG1* and a second closely linked gene.

MATERIALS AND METHODS

Plant lines and growth: All soybean [*Glycine max* (L.) Merr.] seed used in this study was propagated at Harrow, Ontario, Canada. The Flambeau × Merit recombinant inbred lines were derived from a cross between these two cultivars followed by inbreeding to the F_8 generation by single-seed descent.

All plants for pathogen tests were grown in clay pots (4 inch diam) containing a soil:peat:vermiculite:perlite (2:1:0.5:0.5) mix supplemented with osmocote slow-release fertilizer. For the first 2–3 wk after planting, the seedlings were grown in a glasshouse. A photoperiod of ≥16 hr was maintained with supplementary lighting when required. The day before inoculation, plants were transferred to a growth room (16-hr photoperiod, 180 microeinsteins $\cdot m^{-2} \cdot s^{-1}$, 22°).

Bacterial strains and plasmids are described in Table 1.

Growth of bacteria and inoculum preparation: Bacterial lawns were grown on King's medium B (KING *et al.* 1954) supplemented with the appropriate antibiotics at 30° overnight. Rifamycin (Sigma) was included at 100 μg/ml and kanamycin (Sigma) at 50 μg/ml. Bacterial suspensions were prepared from the lawns in 10 mM $MgCl_2$ and diluted to ~1 × 10^8 cfu/ml (an OD_{600} of 0.1) for the HR tests and ~5 × 10^5 cfu/ml for *in-planta* growth analysis. The suspensions were used within 4 hr of preparation.

HR hand-inoculation tests: Primary leaves were inoculated 2–3 wk after planting. The undersides of the leaves were nicked with a razor blade before the inoculum was forced into the apoplast with a 1-ml disposable syringe with no needle fitted. The inoculated panels were scored 20–24 hr after injection. Incompatible (hypersensitive) responses were observed as areas of brown sunken tissue. Typically, no macroscopic response was seen in compatible interactions at this time, although occasionally mild chlorosis was observed. At least five individuals were scored from each recombinant inbred family. Each F_2 individual was injected twice with each bacterial strain being tested.

***In-planta* growth analysis:** *In-planta* bacterial growth analysis was conducted essentially as described by BISGROVE *et al.* (1994). Primary leaves were inoculated when they were fully expanded (2–3 wk after planting). The plants to be inoculated were vacuum infiltrated with an inoculum containing 10 mM $MgCl_2$, 0.001% Silwet L77 surfactant (Osi Specialties, Inc.) and 5 × 10^5 cfu/ml bacteria. A cork borer was used to remove leaf-disc samples from the inoculated leaves 0, 2 and 4 days after inoculation. The bacterial titer in these samples was determined by homogenizing the leaf discs in 10 mM $MgCl_2$ and then plating serial dilutions of the homogenate on trypticase soy agar (Becton Dickinson, Cockeysville, MD) containing 100 μg/ml rifamycin and 50 μg/ml cyclohexamide (Sigma). Colonies were counted after 48 hr. Each datapoint represents the average of four independent samples, and error bars represent one standard error. All *in-planta* bacterial growth analyses were performed at least twice.

Linkage analysis: Map distances in the RI lines were calculated using the Haldane and Waddington equation $p = R/(2 - 2R)$, where p is the frequency of recombinant gametes in a single meiosis and R is the proportion of recombinant individuals. The standard error of p (s_p) was calculated using the formula s_p = the square root of $p(1 - p)/n$, where n is the number of RI lines examined (ALLARD 1956). p and s_p were converted to centimorgans using the

TABLE 2

Resistance of soybean cultivars to *Pseudomonas syringae* pv. *glycinea* race 4 expressing *avrB* or *avrRpm1*

Cultivar/line	Pedigree or origin
H/H = hypersensitive resistant to *avrB*/hypersensitive resistant to *avrRpm1*	
Coles[a]	Hark × (Provar × Disoy × Magna)
Hark[a]	Hawkeye × Harosoy
Hawkeye	Mukden × Richland
Mukden	Hsiao Chin Huang Tou from China
Norchief	Hawkeye × Flambeau
H/S = hypersensitive resistant to *avrB*/susceptible to *avrRpm1*[b]	
AK (Harrow)	Selected from AK (from China)
Blackhawk	Mukden × Richland
Capital	No. 171 × AK (Harrow)
Clark	Lincoln (2) × Richland
Evans	Merit × Harosoy
Harcor	Corsoy (2) × Harosoy 63
Harosoy	Mandarin-Ottawa (2) × AK (Harrow)
Merit	Blackhawk × Capital
Provar	Harosoy × Clark
Richland	PI-70.502-2 (from China)
S/H = susceptible to *avrB*/hypersensitive resistant to *avrRpm1*[c]	
Disoy	[Mandarin (Ott.) × Kanro] × (Richland × Jogun)
Flambeau	Wisc. 839-14
Grande	Anoka × Magna
Jogun	Shirobana from Korea
Kanro	From Korea
Magna	[Mandarin (Ott.) × Jogun] × [Mandarin(Ott.) × Kanro]
Vinton	Hark × (Provar × Disoy × Magna)
S/S = susceptible to *avrB*/susceptible to *avrRpm1*[d]	
Bonminori	PI-360.835 from Japan
CNS	Probably Nanking from China
Higan	Higan Mame from Japan
Peking	From China
Raiden	From Japan
OX615	Harcor (2) × Raiden
OX735	Coles × OX615

Resistance/susceptibility was determined by flooding small areas of the leaf with *P. syringae* pv. *glycinea* race 4 expressing *avrB* or *avrRpm1*. Resistance reactions were scored after 24 hr and disease symptoms monitored after 3 days. H, hypersensitive resistance (HR) response; S, susceptible response.

[a] In some cultivars/lines, the HR response to *avrRpm1* was consistently weak.

[b] Forty tested cultivars/lines not shown.

[c] Fifteen cultivars/lines not shown.

[d] Thirty-six cultivars/lines not shown.

Kosambi function as described by KOORNEEF and STAM (1992). The standard errors on map distances in the F_2 families were calculated using the equation s_p = the square root of $(4 - p^2)/4n$, where n is the number of F_2 individuals examined (ALLARD 1956).

RESULTS

Soybean can distinguish between *avrB* and *avrRpm1*: The observation that the Arabidopsis *RPM1* gene is specific for both *avrB* and *avrRpm1* prompted us to determine whether *avrRpm1*, from the nonhost pathogen *P. syringae* pv. *maculicola* (*Psm*), could be detected by soybean cultivars expressing *RPG1*. One hundred twenty soybean cultivars and lines were hand inoculated with *Psg* race 4 containing *avrB* [*Psg*R4(*avrB*)] or *avrRpm1* [*Psg*R4(*avrRpm1*)] and scored for HRs (*Psg*R4 is virulent on all tested soybean cultivars). Only 5 of the 55 cultivars responding to *avrB* (and so carrying *RPG1*) also responded to *avrRpm1* (Table 2). These were Mukden and four cultivars tracing to it. However, many cultivars lacking a functional *RPG1* gene did respond hypersensitively to *avrRpm1*. Cultivars were also identified that did not respond to either *avrB* or *avrRpm1*. Based on pedigree analysis of the cultivars and lines

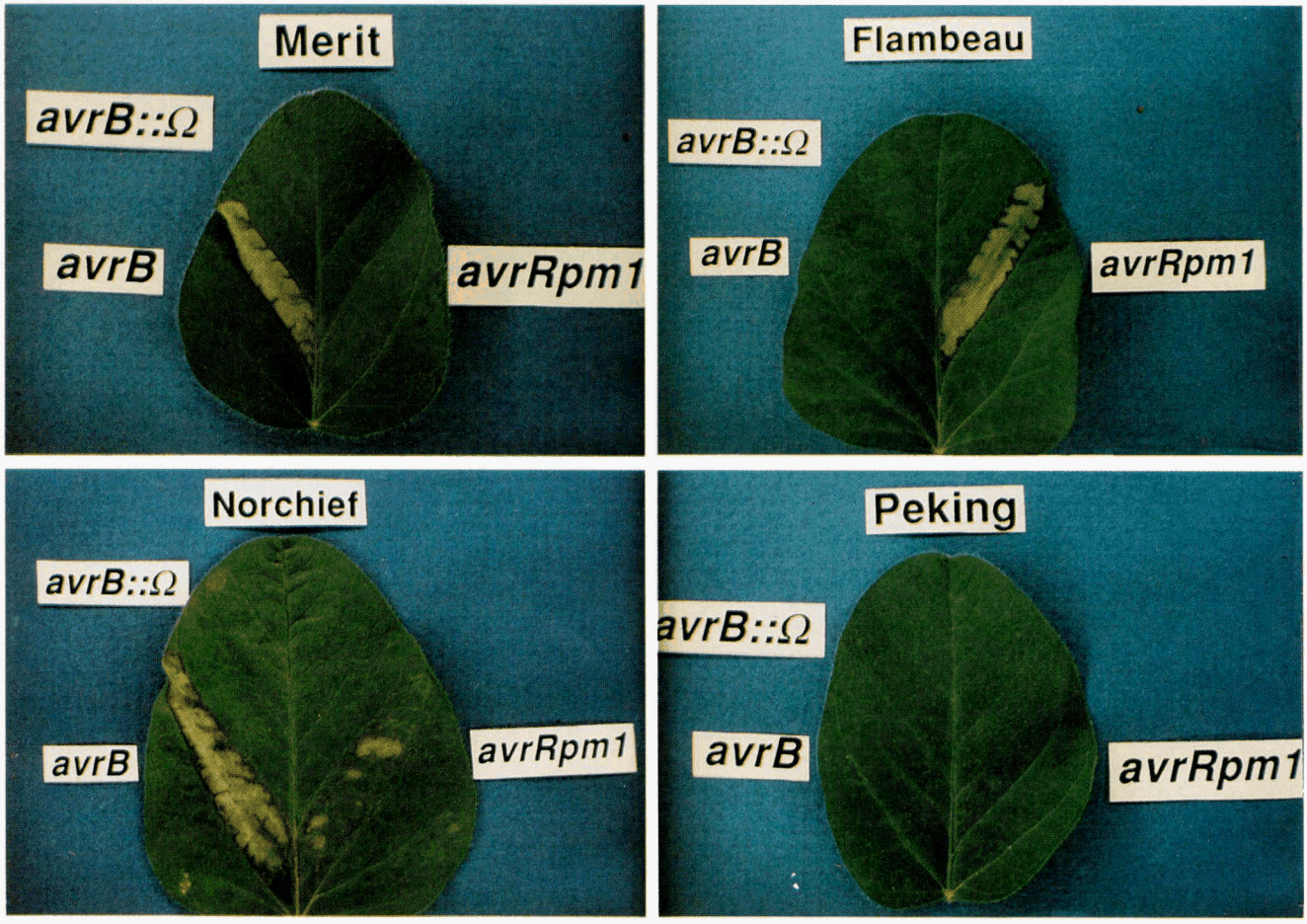

FIGURE 1.—Hypersensitive responses displayed by soybean leaves after interveinal injection with *Pseudomonas syringae* pv. *glycinea* race 4 expressing *avrB* or *avrRpm1*. The primary leaves of 2–3-wk-old soybean plants of cultivars Merit, Flambeau, Norchief and Peking were hand inoculated with *Psg*R4(*avrB*), *Psg*R4(*avrB*::Ω) and *Psg*R4(*avrRpm1*). The leaves were photographed after 24 hr.

(Table 2, and others not shown), cultivar reactions were heritable, that is, each cultivar had at least one parent displaying the same reaction. The cultivars Merit (*avrB* responsive), Flambeau (*avrRpm1* responsive), Norchief (responsive to *avrB* and *avrRpm1*) and Peking (responsive to neither) were chosen as representative of the four classes and selected for further study. The macroscopic responses of these four cultivars to *avrB* and *avrRpm1* are shown in Figure 1.

To confirm that the HR tests accurately reflected the resistance specificities of the four cultivars, *in-planta* bacterial growth was monitored (Figure 2). In all four cultivars, the control *Psg*R4(*avrB*::Ω) strain, which carries *avrB* disrupted with a Ω fragment, multiplied 100–1000-fold over 4 days. The growth of *Psg*R4(*avrB*) in Merit and Norchief and *Psg*R4(*avrRpm1*) in Flambeau and Norchief was severely restricted, reaching a level 50–100-fold less than the *Psg*R4(*avrB*::Ω) control strain. *Psg*R4(*avrRpm1*) consistently multiplied to a lower level in Peking than the control strain. This difference was significant (*t*-test: $t = 3.16$ $P = 0.02$) at day 2 but not at day 4 ($t = 1.43$, $P = 0.20$), and was reproducible over three replicates, suggesting a very weak resistance gene specific for *avrRpm1* in this cultivar. This reduced growth is reflected in attenuated disease symptoms in the infiltrated plants (data not shown) and occasionally led to Peking being scored as *avrRpm1* responsive during the initial cultivar screen. Interestingly, the *Psg*R4(*avrB*) strain displayed a small, but statistically significant, increase in growth over *Psg*R4(*avrB*::Ω) in the *avrB* susceptible cultivars Flambeau ($t = 6.23$, $P = 0.001$ on day 4) and Peking ($t = 3.38$, $P = 0.01$ on day 4). This observation suggests that *avrB* has a role in virulence in compatible interactions.

Resistance to *avrB* and *avrRpm1* resides at the *RPG1* locus: Soybean resistance to *Psg* race 4 expressing *avrB* has been shown previously to be inherited as a single dominant Mendelian trait (KEEN and BUZZELL 1991). The locus responsible has been designated *RPG1* (MUKHERJEE *et al.* 1966). To determine whether resistance to *avrRpm1* is also inherited in a monogenic fashion, the resistance specificities of 95 recombinant inbred lines (RILs) derived from a cross between the cultivars Flambeau and Merit were determined. RILs were chosen for this study because the R-gene specific to *avrRpm1* was found to be incompletely dominant, and we were unable to reliably distinguish individuals heterozygous for this R-gene from homozygous suscep-

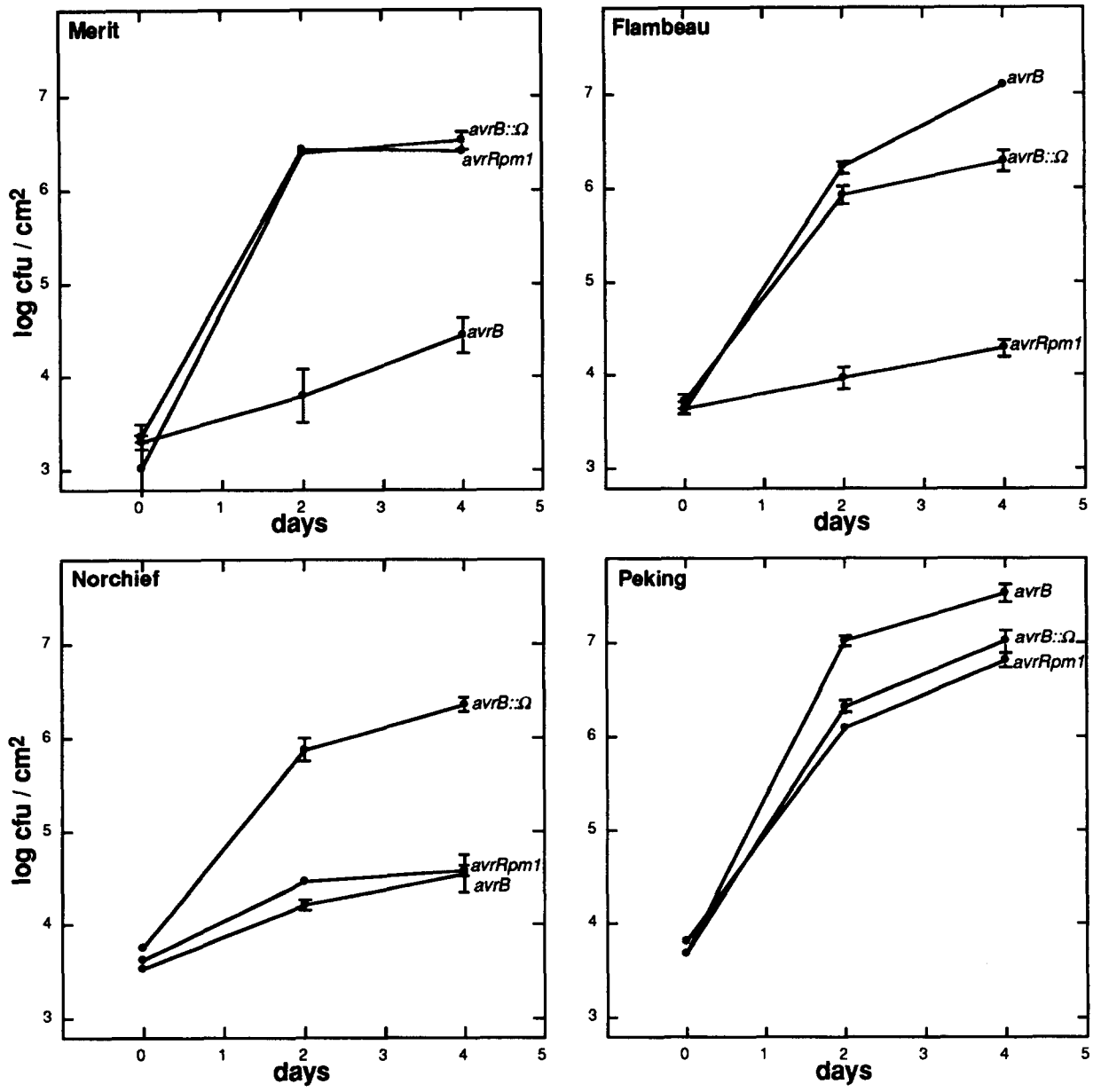

FIGURE 2.—Growth of *Psg* race 4 expressing *avrB* or *avrRpm1* in the leaves of four different soybean cultivars. *Psg* race 4 strains carrying the indicated *avr* genes were vacuum infiltrated into the leaves of 2–3-wk-old soybean plants at a concentration of 5×10^5 cfu/ml. Strain *Psg*R4(*avrB*::Ω) carries *avrB* disrupted with a Ω fragment. At the indicated time points, leaf tissue was removed with a cork borer and the bacterial titer determined. Each data point represents the average of four independent samples and the error bars equal one standard error. cfu, colony forming units.

tibles in F_2 populations. RILs, which are homozygous over most of their genomes, avoid this problem. Resistance/susceptibility to *Psg*R4 (*avrRpm1*) in the Flambeau × Merit RIL population segregated 1:1 ($\chi^2 = 0.17$, $P > 0.5$), indicating the involvement of a single locus that differs between these two cultivars (Table 3).

Because resistance to *avrB* and *avrRpm1* is determined by a single gene in Arabidopsis (GRANT *et al.* 1995), we hypothesized that these two resistance specificities might be controlled by alleles at the *RPG1* locus in soybean. To determine whether genetic linkage exists between the locus responsible for resistance to *Psg*R4(*avrRpm1*) and *RPG1*, we therefore also scored the RI lines for resistance to *Psg*R4(*avrB*). As predicted from the previous study (KEEN and BUZZELL 1991), resistance/susceptibility to *Psg*R4(*avrB*) segregated 1:1 (Table 3; $\chi^2 = 0.38$, $P > 0.5$), confirming the monogenic nature of this resistance. None of the RI lines displayed resistance to both *avr* genes and only a single potential recombinant, susceptible to both *Psg*R4(*avrB*) and *Psg*R4(*avrRpm1*), was detected (line RI-61). These data demonstrate that the resistance specificities for *avrB* and *avrRpm1* are closely linked (0.56 ± 0.77 cM) in soybean. To confirm that family RI-61 represents a true double susceptible, *in-planta* bacterial growth was monitored for both *Psg*R4(*avrB*) and *Psg*R4(*avrRpm1*).

TABLE 3

Segregation of resistance to *Psg* race 4 expressing *avrB* or *avrRpm1* in soybean recombinant inbred families derived from a cross between the cultivars Merit and Flambeau

	No. of families
avrB resistant/*avrRpm1* susceptible	44
avrRpm1 resistant/*avrB* susceptible	49
avrB and *avrRpm1* resistant	0
avrB and *avrRpm1* susceptible	1
families still segregating	1

Resistance phenotype was determined by flooding leaf panels with bacterial suspensions at a concentration of 1×10^8 cfu/ml. Hypersensitive responses were scored 20–30 hr after injection.

Both of these strains were virulent on this genotype (Figure 3). Genotyping of this double susceptible line with four different microsatellite markers revealed only parental alleles (data not shown); thus we found no evidence of a contaminating soybean genotype in this line.

The above data are consistent with resistance to *avrB* and *avrRpm1* in soybean being mediated by two closely linked genes. However, it is possible that alleles at the *RPG1* locus are responsible as we have failed to recombine both specificities onto a single chromosome.

The dual resistance specificity displayed by cultivar Norchief also resides at, or near, the *RPG1* locus: Cultivar Norchief displays resistance to *Psg* race 4 expressing *avrB* or *avrRpm1*. Because this is analogous to the situation observed for Arabidopsis accessions expressing *RPM1*, we hypothesized that a similar dual-specificity allele might be present at the *RPG1* locus. To address this hypothesis, allelism tests were conducted between the *avrB* and *avrRpm1* specificities in Norchief and those in Merit and Flambeau, respectively.

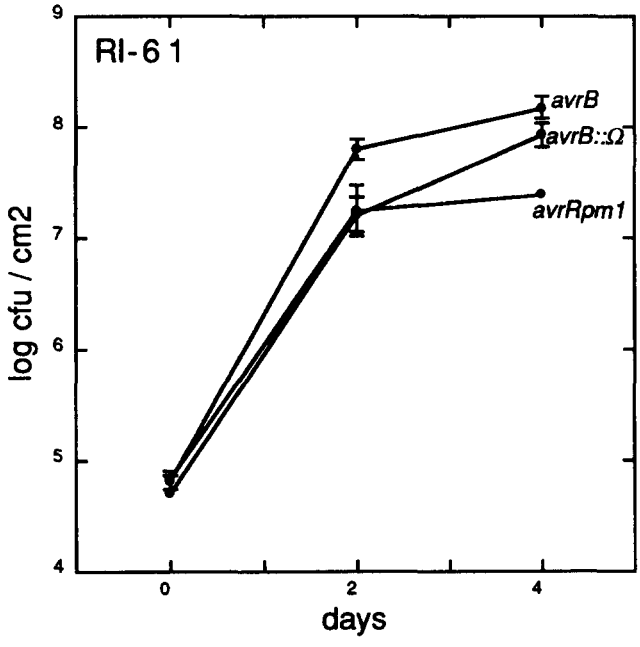

FIGURE 3.—Growth of *Psg* race 4 expressing *avrB* or *avrRpm1* in the leaves of recombinant inbred line 61. *Psg* race 4 strains carrying the indicated *avr* genes were vacuum infiltrated into the leaves of 2–3-wk-old soybean plants at a concentration of 5×10^5 cfu/ml. Strain *Psg*R4(*avrB*::Ω) carries *avrB* disrupted with an Ω fragment. At the indicated time points, leaf tissue was removed with a cork borer and the bacterial titer determined. Each data point represents the average of three independent samples and the error bars equal one standard error. cfu, colony forming units.

Two hundred one F_2 individuals derived from a cross between cultivars Merit and Norchief were scored for their resistance to *Psg*R4(*avrB*) (Table 4). All plants were resistant, demonstrating that the Norchief resistance specificity is allelic, or tightly linked (0 ± 7.0 cM), to that in Merit. We were unable to reliably score this family for resistance to *Psg*R4 (*avrRpm1*) (a large excess of the susceptible class was observed), which we attribute to incomplete dominance of the R-gene relative to *avrRpm1* recognition.

Two hundred fourteen F_2 individuals from the Flambeau × Norchief family were scored for their resistance to *Psg*R4 (*avrRpm1*) (Table 4). No susceptible plants were identified (Table 4), indicating close linkage (0 ± 6.8 cM) between the *avrRpm1* specific R-genes in Norchief and Flambeau. Resistance/susceptibility to *avrB* in this population segregated 3:1 ($\chi^2 = 0.16$, $P > 0.5$), confirming that the resistance to *avrB* displayed by Norchief is mediated by a single dominant locus.

We did not analyze an F_2 family derived from a cross between Norchief × Peking (double susceptible), as the incomplete dominance of the *avrRpm1*-specific R-gene in Norchief rendered such an analysis noninformative without a large amount of progeny testing. However, the incomplete dominance relative to *avrRpm1* strengthens the data obtained from the Flambeau × Norchief cross, as individuals with a single recombinant chromosome that lacked both the Flambeau and Norchief R-genes would likely have been scored as susceptible to *Psg*R4(*avrRpm1*).

These data demonstrate that the dual resistance specificity displayed by Norchief is mediated either by an allele of *RPG1* or by *RPG1* and a second RPG gene closely linked to it.

DISCUSSION

In this study we confirm that some soybean cultivars respond to *avrRpm1* (DANGL *et al.* 1992). Furthermore, we demonstrate that, unlike the situation observed in Arabidopsis, soybean cultivars exist that can distinguish between *avrB* and *avrRpm1*. Cultivars also exist that respond to both *avr* genes, or neither. Finally, by following the segregation of resistance to *avrB* and *avrRpm1* in recombinant inbred and F_2 populations, we demonstrate that these resistance specificities map at, or are tightly linked to, the *RPG1* locus.

That soybean cultivars exist that can distinguish between *avrB* and *avrRpm1* demonstrates that the elicitors

TABLE 4

Segregation of resistance to *Psg* race 4 expressing *avrB* or *avrRpm1* in soybean F_2 populations

F_2 population	*avrB* Resistant	*avrB* Susceptible	*avrRpm1* Resistant	*avrRpm1* Susceptible
Flambeau × Norchief	158	56	214	0
Merit × Norchief	201	0	ND[a]	ND[a]

Resistance phenotype determined as described in Table 2.
[a] Not determined.

produced by these two *avr* genes must be distinct. This was not necessarily to be expected; although these two *avr* genes appear to have unrelated sequences, they are detected (directly or indirectly) by a single R-gene (*RPM1*) in Arabidopsis (BISGROVE *et al.* 1994; GRANT *et al.* 1995). It was theoretically possible that *avrB* and *avrRpm1* directed the production of identical elicitor molecules. *RPM1* must therefore code for a receptor able to detect two distinct elicitors or for a component of a signal transduction pathway used by receptors specific for the *avrB* and *avrRpm1* elicitors.

The distinction between the *avrB* and *avrRpm1* elicitors was also apparent in compatible interactions in soybean as only *avrB* appeared to contribute to virulence. That *avrB* should act as a virulence factor but not *avrRpm1* is intriguing because during compatible interactions between *Psm* and Arabidopsis, the inverse is true (RITTER and DANGL 1995). This is perhaps not surprising as *avrB* originates from *Psg* (a soybean pathogen) and *avrRpm1* originates from *Psm* (an Arabidopsis pathogen). These observations are consistent with previous evidence that demonstrated that bacterial virulence factors can be host specific in their action (SWARUP *et al.* 1991; DE FEYTER *et al.* 1993; RITTER and DANGL 1995).

Our results indicate that soybean resistance to *Psg* strains expressing *avrB* or *avrRpm1* is mediated by alleles of *RPG1* or by *RPG1* and a second closely linked gene. When a cultivar–race series exists between a crop plant and a fungal pathogen, resistance genes corresponding to different races of the pathogen are often clustered either as closely linked genes or as alleles (reviewed by PRYOR and ELLIS 1993). For example, the maize *Rp1* locus contains numerous tightly linked R-genes corresponding to specific races of the rust pathogen *Puccinia sorghi* (HULBERT and BENNETZEN 1991). In contrast, the available evidence suggests that the multiple resistance genes corresponding to races of the flax rust *Melampsora lini* are alleles at the *L* locus as it has not been possible to recombine two specificities onto the same chromosome (ISLAM *et al.* 1989).

Clustering of R-genes specific to bacterial pathogens appears to be uncommon, however. The four previously identified R-genes in soybean that are specific to *P. syringae* avirulence genes are not closely linked (KEEN and BUZZELL 1991). Likewise, none of the four R-genes in Arabidopsis specific to *P. syringae* avirulence genes are linked (DEBENER *et al.* 1991; KUNKEL *et al.* 1993; HINSCH and STASKAWICZ 1995; SIMONICH and INNES 1995). The only examples of linked bacterial resistance genes of which we are aware are *Xa-10* and *Xa-4* (YOSHIMURA *et al.* 1983),and *Pto1* and *Pto2* (STOCKINGER and WALLING 1994). *Xa-10* and *Xa-4* are rice genes that confer resistance to races of *Xanthomonas oryzae* and are ~27 cM apart. *Pto1* and *Pto2* are tomato genes that confer resistance to specific races of *P. syringae* pathovar *tomato* and are reported to be within 9 cM of each other, but no linkage data have been published. There are no reports of complex R-gene loci specific for bacterial *avr* genes analogous to the *Rp1* or *L* loci.

We identified one potential recombinant family among 95 RI lines segregating for resistance to *Psg*R4(*avrB*) and *Psg*R4(*avrRpm1*). The simplest explanation for this observation is that resistance to *avrB* and *avrRpm1* in soybean is controlled by two tightly linked genes. It is possible, however, that both resistance specificities are mediated by alleles and that the double-susceptible family resulted from intragenic recombination, unequal crossing over, or transposon-induced mutation. These processes have been proposed to account for the recovery of double-susceptible progeny from individuals heterozygous for distinct "alleles" at the *L* locus in flax (ISLAM *et al.* 1989). The dual specificity displayed by the soybean cultivar Norchief could be mediated by an *RPG1* allele able to respond to both *avrB* and *avrRpm1*. This is a plausible hypothesis as it has been shown that the Arabidopsis *RPM1* gene responds to both these *avr* genes, demonstrating that R-genes may have multiple specificities (BISGROVE *et al.* 1994; GRANT *et al.* 1995).

RPM1 has recently been cloned (GRANT *et al.* 1995) and shown to contain motifs conserved in other R-genes corresponding to bacterial, fungal and viral pathogens (reviewed by BRIGGS 1995; DANGL 1995; INNES 1995; STASKAWICZ *et al.* 1995). We are now focused on cloning *RPG1* and the allele/linked gene specific for *avrRpm1*. Comparison of these soybean alleles/genes to each other and to *RPM1* may provide valuable information on how specificity is conferred to R-genes.

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