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RESEARCH ARTICLE

# Expression of the Tomato *Pto* Gene in Tobacco Enhances Resistance to *Pseudomonas syringae* pv *tabaci* Expressing *avrPto*

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**The *Pto* gene encodes a serine–threonine kinase that confers resistance in tomato to *Pseudomonas syringae* pv *tomato* strains expressing the avirulence gene *avrPto*. We examined the ability of *Pto* to function in tobacco, a species that is sexually incompatible with tomato. Evidence that a heterologous *Pto*-like signal transduction pathway is present in tobacco was suggested by the fact that tobacco line Wisconsin-38 exhibits a hypersensitive response after infection with *P. syringae* pv *tabaci* expressing *avrPto*. We introduced a *Pto* transgene into cultivar Wisconsin-38 and assessed the ability of transformed plants to further inhibit growth of the *P. s. tabaci* strain expressing *avrPto*. The *Pto*-transformed tobacco plants exhibited a significant increase in resistance to the avirulent *P. s. tabaci* strain compared with wild-type tobacco as indicated by (1) more rapid development of a hypersensitive resistance response at high inoculum concentrations ($10^8$ colony-forming units per mL); (2) lessened severity of disease symptoms at moderate inoculum concentrations ($10^6$ and $10^7$ colony-forming units per mL); and (3) reduced growth of avirulent *P. s. tabaci* in inoculated leaves. The results indicate that essential components of a *Pto*-mediated signal transduction pathway are conserved in tobacco and should prompt examination of resistance gene function across even broader taxonomic distances.**

## INTRODUCTION

Genes that confer resistance to pathogenic microbes occur in most economically important plant species (Allard, 1960; Nelson, 1973; Singh, 1986). In many cases, these genes play important roles in limiting crop losses resulting from diseases and in reducing the need for pesticides (Nelson, 1973; Singh, 1986). Plant breeders frequently identify disease resistance (*R*) genes in wild relatives or closely related species of cultivated plants and, when possible, introgress these genes into agronomic cultivars by backcrossing (Allard, 1960; Knott and Dvorak, 1976; Knott, 1987). From this research, we have learned that certain *R* genes function when transferred among closely related plant species (Knott and Dvorak, 1976). However, backcrossing requires that two species be sexually compatible, and this has limited the ability to transfer *R* genes across broader taxonomic distances. The recent molecular cloning of *R* genes offers the potential to introduce these important genes into unrelated crop species using transformation technology (Keen et al., 1993; Moffat, 1994). This strategy could greatly expand the number of *R* genes available to plant breeders (Keen et al., 1993). However, whether *R* genes isolated from one species will function when introduced into sexually incompatible plant species remains to be determined.

Many *R* genes act by recognizing the presence of a specific avirulence (*avr*) gene in the pathogen (Keen, 1990). Numerous *avr* genes have been cloned from bacterial and fungal pathogens, and in most cases, their biochemical roles remain unclear (Dangl, 1994). Recently, several *R* genes have been isolated from plants that respond to the expression of specific *avr* genes (Martin et al., 1993b; Bent et al., 1994; Jones et al., 1994; Lawrence et al., 1994; Mindrinos et al., 1994; Whitham et al., 1994). These *R* genes encode proteins that appear to be involved in two different aspects of pathogen recognition and signal transduction. Proteins of one class contain a region of leucine-rich repeats (LRR) that may be directly involved in protein or ligand binding (Bent et al., 1994; Jones et al., 1994; Lawrence et al., 1994; Mindrinos et al., 1994; Whitham et al., 1994). One *R* gene product of the LRR type contains a domain that is similar to the Drosophila Toll protein and the mammalian interleukin-1 receptor protein (Whitham et al., 1994). The second type of *R* gene is represented by *Pto*, which confers resistance in tomato to *Pseudomonas syringae* pv *tomato* strains that express *avrPto* (Martin et al., 1993b). *Pto* encodes a functional serine–threonine kinase that possesses a putative myristoylation site at its N terminus (Martin et al., 1993b; Loh and Martin, 1995a; Rommens et al., 1995). When inoculated with a high concentration of *P. s. tomato* ($10^8$ colony-forming units [cfu]/mL), resistant tomato plants (*Pto*/–)

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exhibit a hypersensitive resistance response manifested as rapid cell death (<24 hr after inoculation) at the point of infection. When inoculated with lower concentrations of bacteria, leaves of *Pto*-containing tomato plants exhibit no macroscopic symptoms. Leaves of susceptible tomato plants (*pto/pto*) develop disease symptoms 3 to 5 days after infection. These symptoms consist of necrosis at the point of inoculation surrounded by chlorosis (Martin et al., 1993a).

Hybridization experiments with the cloned *Pto* gene as a probe have revealed that homologs of this gene are present in all plant species examined, including potato, tobacco, Arabidopsis, soybean, and various cereals (Martin et al., 1993b). These observations raised the possibility that a disease resistance mechanism involving a *Pto* homolog may exist in widely divergent plant species. Evidence for a gene that has a similar function to *Pto* was obtained with soybean when cultivar Centennial was found to develop a hypersensitive response specifically to *P. syringae* pv *glycinea* strains expressing *avrPto* (Ronald et al., 1992). There are many other similar examples in which the expression of an *avr* gene isolated from one bacterial pathogen is recognized by a plant species that is not normally a host to that pathogen (Whalen et al., 1988, 1991; Kobayashi et al., 1989; Dangl et al., 1992). Collectively, these observations suggest that homologous *R* genes and the signal transduction pathways in which they participate may be widely conserved in the plant kingdom.

In this study, we developed transgenic tobacco plants that contain an integrated copy of the tomato *Pto* gene. The transgenic plants were compared with the wild-type progenitor tobacco line to examine the effect of the *Pto* transgene on hypersensitive response, disease development, and growth of the bacterial pathogen in leaves.

## RESULTS

### Transfer of *avrPto* to *P. syringae* pv *tabaci* and *Pto* to Tobacco

We chose tobacco (*Nicotiana tabacum* cv Wisconsin-38 [W-38]) to test for cross-species function of the tomato *Pto* gene because (1) it is easily transformed by Agrobacterium; (2) its large leaves lend themselves to inoculation experiments; and (3) tobacco and tomato are sexually incompatible species that belong to the same plant family (Solanaceae). Because soybean, a more distantly related plant species, has already been shown to possess a functional homolog of *Pto* (Ronald et al., 1992), we also decided to determine whether tobacco contains a functional *Pto* homolog.

Fourteen putative tobacco transformants ($R_0$) were produced by Agrobacterium-mediated transformation of line W-38 by using the *Pto* transgene construct pPTC8 (see Methods). Eight of the $R_0$ plants exhibited altered disease symptoms, compared with the W-38 control, when inoculated with *P. s. tabaci* expressing *avrPto*. Progeny derived from self-fertilization of four of the $R_0$ plants segregated in a ratio of ~3:1 for resistance/sensitivity to kanamycin, indicating that the transgene was integrated at a single locus in each of these plants. In all cases, resistance to kanamycin was perfectly correlated with the altered reaction to the *P. s. tabaci* strain expressing *avrPto* described below. This correlation indicates that the altered disease symptoms are attributable to expression of the *Pto* transgene. Three independent, homozygous $R_1$ plants (Tb-1, Tb-2, and Tb-3) were identified by testing their progeny for germination on kanamycin medium and by DNA gel blot analysis. The $R_2$ homozygous progeny from these plants were used in subsequent experiments, and the lines are referred to by the designation of their respective $R_1$ parent line. DNA gel blot analysis of Tb-1, Tb-2, and W-38 plants is shown in Figure 1. Consistent with previous observations, wild-type tobacco line W-38 contains several DNA sequences homologous with *Pto* (Figure 1; Martin et al., 1993b).

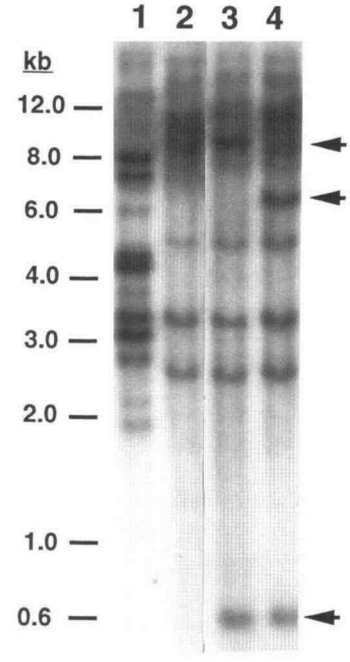


**Figure 1.** DNA Gel Blot Analysis of Tobacco Lines Transformed with the 35S::*Pto* Transgene.

Genomic DNA was digested with SpeI (3 μg for tomato and 5 μg for tobacco), separated on a 1% agarose gel, and transferred to a Hybond $N^+$ filter. The filter was probed with a radiolabeled *Pto* polymerase chain reaction product. Lane 1 contains tomato DNA; lane 2, wild-type tobacco W-38 DNA; lane 3, *Pto*-transformed tobacco Tb-1 DNA; and lane 4, *Pto*-transformed tobacco Tb-2 DNA. Tb-1 and Tb-2 are independent transformants. Arrowheads indicate fragments in the transgenic plants that correspond to the introduced *Pto* transgene. The band at 0.6 kb represents a *Pto* internal fragment, and the 6.4- and 9-kb bands are junction fragments between the transgene and tobacco genomic DNA. Locations of length standards in kilobases are indicated at left.

*P. s. tabaci*, the causal agent of wildfire disease in tobacco, was chosen for this study because its pathogenicity is well characterized, and a strain (11528R, race 0) highly virulent on tobacco line W-38 was readily available (Durbin, 1979; Knoche, 1990). Plasmid pPtE6 containing *avrPto* was introduced into strain 11528R by triparental mating, and transconjugants were confirmed by DNA gel blot analysis (data not shown). Although many *P. syringae* strains contain sequences homologous with *avrPto* (Ronald et al., 1992), *P. s. tabaci* 11528R does not (R.L. Thilmony and G.B. Martin, unpublished results).

Our experimental design re-created the tomato *Pto–avrPto* gene-for-gene interaction in the tobacco–*P. s. tabaci* system and addressed two fundamental questions. First, is there a functional homolog of *Pto* in wild-type tobacco that recognizes the expression of *avrPto* in *P. s. tabaci*? This plant–pathogen combination was designated interaction 2. Second, does expression of the tomato *Pto* gene in tobacco enable the plant to detect the presence of the *avrPto* gene in *P. s. tabaci* (or enhance this ability if a functional *Pto* already exists in tobacco)? We designated this gene-for-gene recognition event as interaction 1. Observations of interactions 1 and 2 address the question, Is a *Pto*-mediated signal transduction pathway conserved in tobacco? Controls for the compatible interaction involving *P. s. tabaci* lacking *avrPto* were designated interaction 3 for *Pto*-transformed plants and interaction 4 for wild-type tobacco (Durbin, 1979; Knoche, 1990).

### Hypersensitive Resistance in *Pto*-Transformed and Wild-Type Tobacco Plants

The hypersensitive resistance response is characterized by rapid collapse and death (usually within 12 to 24 hr) of the host tissue precisely confined to the inoculated area (Goodman and Novacky, 1994). Following desiccation of the tissue, it becomes brown and necrotic. Hypersensitivity indicates either gene-for-gene resistance or non-host resistance. In a hypersensitive reaction, bacterial populations decline and surviving bacteria are confined to the inoculation site. For example, resistant tomato plants (*Pto*/–) exhibit a hypersensitive response within 24 hr when inoculated with high titers (>$10^8$ cfu/mL) of *P. s. tomato* containing *avrPto*, and bacterial growth is inhibited ~100-fold in comparison with susceptible tomato plants.

To determine whether the *Pto* transgene will confer a gene-for-gene hypersensitive resistance response in tobacco, fully expanded leaves from 7-week-old Tb-2 and W-38 plants were infiltrated with $10^8$ cfu/mL of *P. s. tabaci* or *P. s. tabaci* expressing *avrPto* and assayed by visual inspection. As shown in Figure 2, the earliest response occurred in interaction 1, when, 10 to 13 hr after inoculation, a hypersensitive response was observed as translucence and collapse of the infiltrated area. At this time, no symptoms were visible in any other interaction. At 18 to 20 hr after inoculation, a similar hypersensitive response was seen in interaction 2, with the infiltrated area becoming collapsed and translucent (Figure 2). Symptoms did not appear in the compatible interactions 3 or 4 until 27 to 30 hr after inoculation, when localized necrosis developed within the infiltrated area (Figure 2). The more rapid development of the hypersensitive response in interaction 1 was observed in numerous experiments with each of the independent transgenic lines Tb-1, Tb-2, and Tb-3.

### Disease Symptoms at Lower Inoculum Concentrations

In tomato plants containing the *Pto R* gene, inoculum of avirulent *P. s. tomato* at concentrations <$10^8$ cfu/mL does not elicit a macroscopic hypersensitive response. To test whether a similar dose-dependent response would be exhibited by *Pto*-transformed tobacco, we conducted an inoculum titration experiment. Cultures of *P. s. tabaci* (with and without *avrPto*) were grown overnight and resuspended at 10-fold serial dilutions ranging from $10^8$ to $10^3$ cfu/mL. Aliquots of each dilution were infiltrated into adjacent regions delimited by the lateral veins of Tb-1 and W-38 tobacco leaves, as shown in Figure 3.

Symptoms were monitored at 24-hr intervals and photographed 7 days after inoculation (Figure 3). Compatible interactions 3 and 4 produced disease symptoms after inoculation with *P. s. tabaci* strains at concentrations of $10^5$ to $10^8$ cfu/mL (Figure 3). In these interactions, symptoms developed gradually, beginning at 30 hr postinoculation, and ultimately resulted in necrosis of the entire infiltrated area surrounded by mild chlorosis. Minor leaf necrosis was observed with bacterial concentrations at $10^4$ cfu/mL. In contrast, the symptoms were strikingly different in interactions 1 and 2. In interaction 1, no leaf necrosis was observed at bacterial concentrations ranging from $10^3$ to $10^6$ cfu/mL, and only mild chlorosis was seen with inoculum of $10^7$ cfu/mL (Figure 3). As expected in interaction 1, a strong hypersensitive response was observed at $10^8$ cfu/mL, beginning at 10 hr postinoculation. Seven days after inoculation in interaction 1, necrosis similar to the compatible interactions was observed; however, there was no spreading chlorosis surrounding the infiltrated area. Responses in interactions 1 and 2 to bacterial concentrations ranging from $10^3$ to $10^5$ cfu/mL were similar, with no obvious symptoms developing after inoculation. However, in interaction 2, an inoculum level of $10^6$ cfu/mL and especially $10^7$ cfu/mL resulted in gradual symptom development similar to interactions 3 and 4. As before in interaction 2, infiltration with $10^8$ cfu/mL produced a hypersensitive response 20 hr after inoculation. Identical results were obtained using transgenic lines Tb-2 and Tb-3.

Similar experiments were performed with a derivative of 11528R that does not produce the phytotoxin tabtoxin (Knoche, 1990). Although disease symptoms were diminished with bacterial concentrations below $10^6$ cfu/mL and there was less chlorosis, the temporal expression of symptoms was identical to that observed in response to wild-type strain 11528R (data not shown).

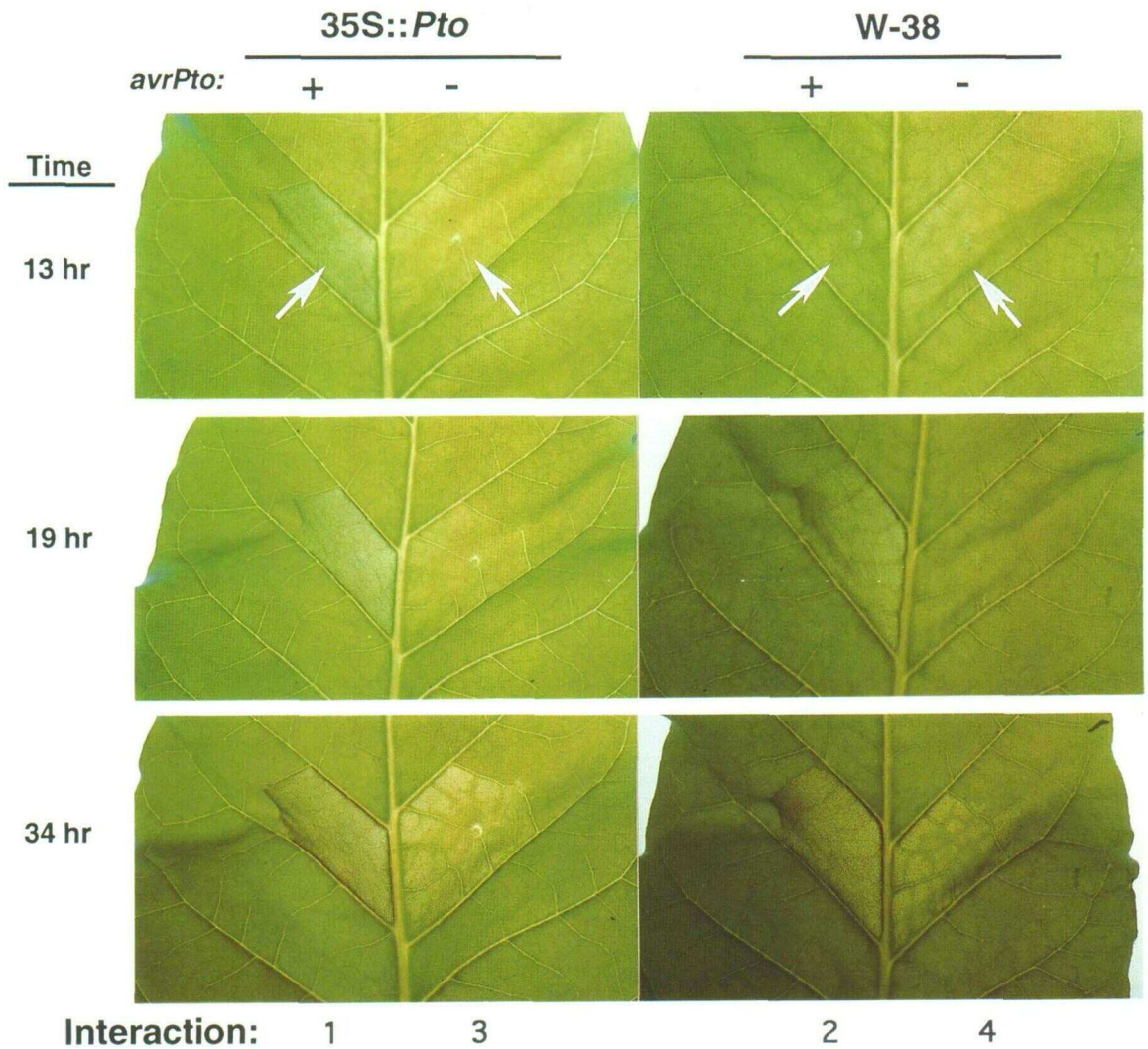


**Figure 2.** Hypersensitive Resistance Response in *Pto*-Transformed Tobacco.

**(Left)** Fully expanded leaves from 7-week-old plants of tobacco line Tb-2 (homozygous 35S::*Pto*).
**(Right)** Fully expanded leaves from 7-week-old plants of wild-type tobacco W-38.
Plants were inoculated with *P. s. tabaci* strain 11528R expressing *avrPto* (+) or *P. s. tabaci* strain 11528R lacking *avrPto* (–). An equal volume (50 μL) of $10^8$ cfu/mL of the pathogen was infiltrated into a region between lateral veins (see arrows). Photographs were taken of leaves remaining on the plant and show the progression of symptoms on a single representative leaf from each tobacco line at 13, 19, and 34 hr after inoculation. Interactions are designated 1 (*P. s. tabaci* 11528R expressing *avrPto*/W-38 tobacco expressing 35S::*Pto*), 2 (*P. s. tabaci* 11528R expressing *avrPto*/W-38 tobacco), 3 (*P. s. tabaci* 11528R lacking *avrPto*/W-38 tobacco expressing 35S::*Pto*), and 4 (*P. s. tabaci* 11528R lacking *avrPto*/W-38 tobacco).

### Growth of *P. s. tabaci* in Plant Leaves

To determine whether the hypersensitive response and disease symptoms reflect the degree of bacterial growth, we monitored *P. s. tabaci* populations in leaves of W-38 and *Pto*-transformed tobacco plants. Leaves were syringe infiltrated with *P. s. tabaci* or *P. s. tabaci* expressing *avrPto* at a concentration of $10^6$ cfu/mL, and bacterial growth was monitored over a 5-day period as summarized in Figure 4. An inoculum concentration of $10^6$ cfu/mL was used because differential symptom expression occurred between the compatible interactions and the two incompatible interactions at this inoculum density (Figure 3). In these experiments, bacterial growth was reduced eight- to 70-fold in interaction 1 in comparison with interaction 2, and 30- to 300-fold in comparison with compatible interactions 3 and 4 over the course of the experiment. The increase in bacterial

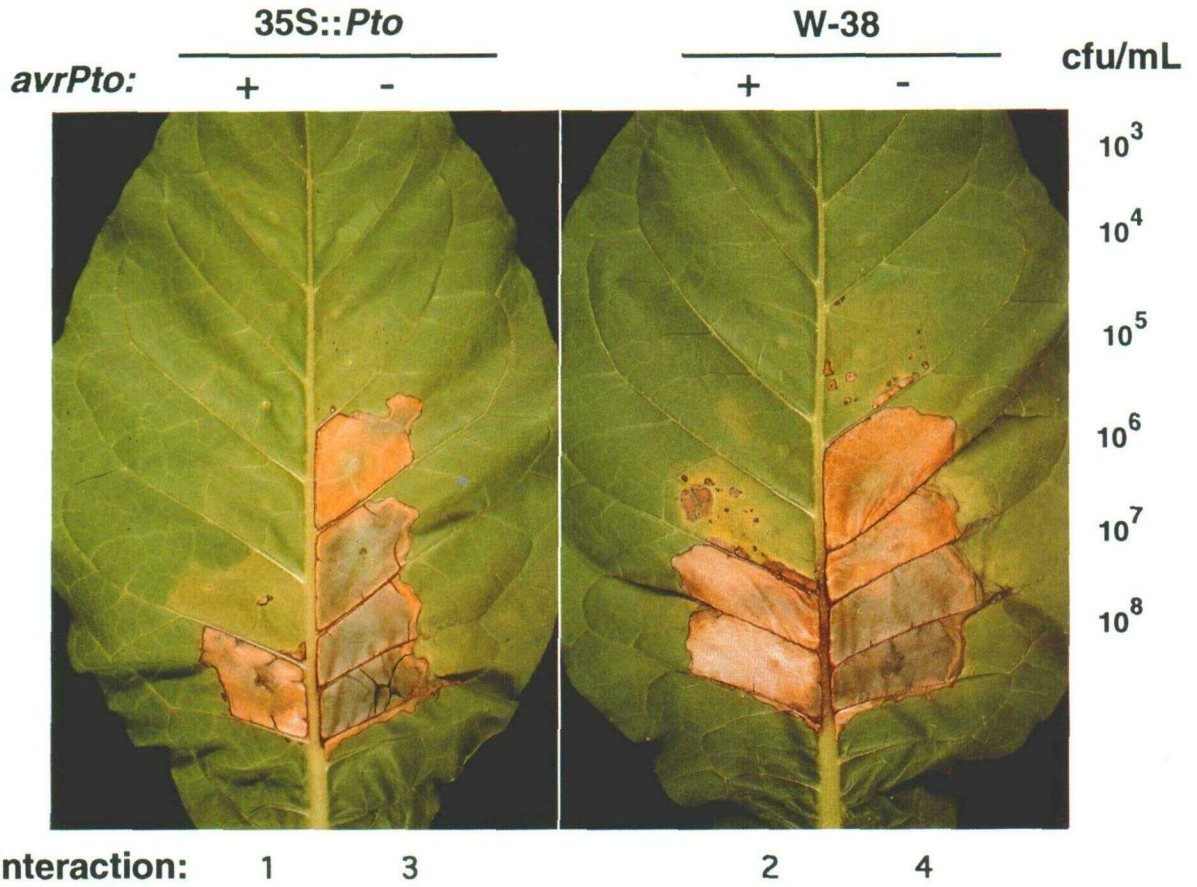


**Figure 3.** Reaction of Tobacco Lines to Various Inoculum Concentrations of *P. s. tabaci* Strains.

**(Left)** Fully expanded leaf from a 7-week-old plant of tobacco line Tb-1 (homozygous 35S::*Pto*).
**(Right)** Fully expanded leaf from a 7-week-old plant of wild-type tobacco W-38.
Symptoms are shown for transgenic and wild-type plants after inoculation with various concentrations of *P. s. tabaci* expressing *avrPto* (left half of leaves) or with *P. s. tabaci* lacking *avrPto* (right half of leaves). Regions between the lateral veins were inoculated, as described in Methods, with the following (beginning from the bottom): $10^8$, $10^7$, $10^6$, $10^5$, $10^4$, or $10^3$ cfu/mL. Interaction designations 1, 2, 3, and 4 are as given in the legend to Figure 2. Photographs were taken 7 days after inoculation.

growth in interaction 2 was intermediate between that of interaction 1 and interactions 3 and 4, and ranged from three- to eightfold less than in the compatible interactions (Figure 4).

## DISCUSSION

The recent cloning of genes that confer resistance to various bacterial, fungal, and viral pathogens opens the way for the molecular manipulation and transfer of these genes to taxonomically diverse plant species. However, the taxonomic range over which *R* genes function is currently an open question. Introgression of *R* genes among cereal species by conventional breeding indicates that disease resistance mechanisms by which *R* genes operate are conserved among closely related, sexually compatible species (Knott and Dvorak, 1976; Knott, 1987). However, until *R* genes were cloned, it was not possible to address the question of *R* gene function among more diverse, sexually incompatible species. In the work presented here, we used a cloned *R* gene from tomato to assess its ability to function in a related but sexually incompatible plant species, tobacco.

To test the function of tomato *Pto* in tobacco, we transferred the gene into tobacco line W-38 and its corresponding avirulence gene, *avrPto*, into *P. s. tabaci*, a natural tobacco leaf pathogen. By using this heterologous gene-for-gene system, we were able to address the following two questions: Does tobacco contain a functional *Pto* homolog? Will the tomato *Pto* gene function in a sexually incompatible species such as tobacco? We believed it was important to test the latter question by using a plant species that is closely related to tomato, because in such a species, any additional mechanisms needed for *Pto* function might also be conserved.

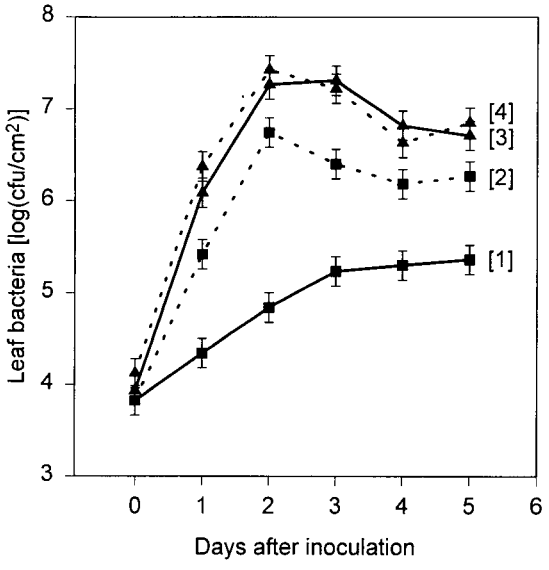


**Figure 4.** Growth of *P. s. tabaci* Strains in Plant Leaves.

Growth of *P. s. tabaci* in leaves of *Pto*-transformed tobacco line Tb-2 (solid lines) and wild-type tobacco W-38 (dashed lines) is shown. Fully expanded leaves of 9-week-old tobacco plants were inoculated with $10^6$ cfu/mL of a *P. s. tabaci* strain expressing *avrPto* (squares) or lacking *avrPto* (triangles). Bacterial populations were determined at the specified time points, as described in Methods. Interaction designations 1, 2, 3, and 4 are indicated and are as given in the legend to Figure 2. Values are the means of three samples, each consisting of three leaf discs from three different plants. Error bars equal one-half of the least significant difference at a probability level of 0.05. Means are significantly different where error bars do not overlap.

### A Functional Homolog of *Pto* in Wild-Type Tobacco

DNA sequences that are homologous with *Pto* occur in many plant species, including tobacco (Martin et al., 1993b). The presence of *Pto* structural homologs in diverse species suggests that similar disease resistance pathways might be widely conserved in the plant kingdom. However, it is not known whether these structural homologs actually correspond to functional *R* genes.

The results presented here show that a functional homolog of *Pto* exists in wild-type W-38 tobacco. Three lines of evidence support this conclusion. First, a macroscopic hypersensitive response was exhibited in interaction 2, beginning 18 to 20 hr after inoculation with avirulent *P. s. tabaci.* The hypersensitive reaction occurred at least 10 hr prior to the appearance of any disease symptoms in the corresponding compatible interaction (interaction 4). This *avrPto*-specific response could not have been due to reduced fitness of the *P. s. tabaci* strain expressing *avrPto* and hence provides strong support for the presence of a functional *Pto* homolog in tobacco. Second, bacterial growth in interaction 2 was reduced compared with compatible interaction 4. This growth inhibition could be explained by decreased fitness of the pathogen as a result of *avrPto* expression in this strain. However, we believe this possibility is unlikely because we have seen no effect on symptom development in susceptible tomato leaves when a *P. s. tomato* strain expressing *avrPto* was used in comparable experiments (G.B. Martin, unpublished results). In addition, other researchers have observed similar levels of bacterial growth in susceptible tomato leaves inoculated with *P. syringae* strains with or without *avrPto* (Carland and Staskawicz, 1993). Based on these observations, we believe it is unlikely that the lower bacterial populations seen in leaves are due to decreased pathogen fitness. Rather, we believe that they reflect recognition of *avrPto* by an endogenous *Pto*-like activity in tobacco. Finally, disease symptoms elicited at inoculum concentrations below $10^8$ cfu/mL were markedly decreased in interaction 2 as compared with interaction 4, indicating specific recognition and defense against the *avrPto*-containing *P. s. tabaci* strain.

By crossing W-38 to a tobacco line that does not possess *Pto*-like activity, it should be possible to determine by genetic analysis whether a structural *Pto* homolog in W-38 cosegregates with the tobacco *Pto*-like resistance. Alternatively, isolation of tobacco *Pto* homologs and transformation of these genes into susceptible tomato plants would show conclusively whether a tobacco *Pto* homolog is functional. To address this latter possibility, we recently isolated a *Pto* homolog from tobacco W-38 and showed that it encodes a protein that has 80% conserved amino acids when compared with the tomato *Pto* gene product (R. Frederick and G.B. Martin, unpublished results).

### Function of the Tomato *Pto* Gene in Tobacco

We demonstrated that the tomato *Pto* gene functions in transgenic tobacco lines and significantly increases the level of gene-for-gene resistance to *P. s. tabaci* expressing *avrPto.* Compared with wild-type tobacco, transgenic tobacco plants showed (1) more rapid development of a hypersensitive response at high bacterial concentrations ($10^8$ cfu/mL); (2) enhanced suppression of disease symptoms when infected with inoculum concentrations of $10^6$ and $10^7$ cfu/mL; and (3) up to a 70-fold reduction of bacterial growth in infected leaves. Additional support for conservation of a *Pto*-mediated pathway in a heterologous species is provided by the demonstration of *Pto* function in another tobacco species, *N. benthamiana* (Rommens et al., 1995b).

The more rapid hypersensitive response seen in *Pto*-transformed plants might have been due to overexpression of the tomato *Pto* gene by the cauliflower mosaic virus 35S promoter. Alternatively, the tomato *Pto* protein might be more effective in recognizing expression of *avrPto.* In either case, these results clearly imply that the protein kinase encoded by *Pto* is able to utilize a tobacco signal transduction pathway to confer higher levels of resistance to *P. s. tabaci* expressing *avrPto.* It is likely that components of this signaling pathway

are homologous with components in tomato. However, it is also possible that *Pto* function in tobacco could be mediated by different factors in tobacco.

The enhanced resistance phenotype conferred by the tomato *Pto* gene in tobacco indicates that the function of essential components of a *Pto* disease resistance signaling pathway is conserved in tobacco. Possible components of a *Pto*-mediated signal transduction pathway might include a transmembrane receptor, other kinases, phosphatases, and transcriptional activators of various defense genes. Recently, mutagenesis experiments have identified another gene in tomato, *Prf*, that is involved in expression of both *P. s. tomato* resistance and fenthion sensitivity (Salmeron et al., 1994). Our results indicate that if *Prf* is required for *Pto* function, a homolog of this gene probably exists in tobacco. The availability of *Pto*-transformed tobacco plants and the ease with which this species is transformed by Agrobacterium will be useful when examining additional candidate genes in this pathway by using overexpression and antisense RNA technology or dominant negative suppression of tobacco gene function.

The finding that an *R* gene from tomato functions in tobacco, a sexually incompatible species, supports the hypothesis that mechanisms involved in specific gene-for-gene systems are conserved within the Solanaceae and quite possibly over broader taxonomic distances. Additional experiments with *Pto* and with *R* genes from the LRR class are necessary to determine the extent to which *R* genes will function in even more taxonomically divergent plant species.

## METHODS

### Tobacco Transformation

A cDNA insert of *Pto* was placed into the binary Ti vector pBI121 (Clontech Laboratories, Palo Alto, CA) in the sense orientation. The transgene was under the transcriptional control of the cauliflower mosaic virus 35S promoter (Martin et al., 1993b). This construct, designated pPTC8, confers resistance in tomato plants to *Pseudomonas syringae* pv *tomato* strains expressing *avrPto* (Martin et al., 1993b). The plasmid pPTC8 was electroporated into *Agrobacterium tumefaciens* LBA4404, and the bacteria were used to transform leaf explants of *Nicotiana tabacum* cv Wisconsin-38 (W-38) as previously described (Horsch et al., 1985). $R_1$ progeny from putative independent transformants that segregated 3:1 (resistant to sensitive) when germinated on kanamycin-containing medium were transferred to soil and grown for seed. Homozygous $R_1$ plants containing an integrated copy of the T-DNA were identified by progeny tests and DNA gel blot analysis, and their homozygous $R_2$ progeny (35S::*Pto*/35S::*Pto*) were used in additional experiments.

### Bacterial Strains, Plasmids, and Triparental Mating

The plasmid pPtE6 containing avirulence gene *avrPto* (Ronald et al., 1992) was introduced into *P. syringae* pv *tabaci* strain 11528R race 0 using triparental mating (Willis et al., 1988). Plasmid pPtE6 was similarly introduced into *P. s. tabaci* 11528R(Δ[TβL]2), a spontaneous deletion mutant of 11528R that has lost the tabtoxin biosynthetic region (Knoche, 1990).

### Bacterial Inoculation, Bacterial Growth Measurements in Leaves, and Plant Growth Conditions

Cells of *P. s. tabaci* strains with or without the plasmid pPtE6 (*avrPto*; Ronald et al., 1992) were grown overnight in King's medium B (KMB; Martin et al., 1993b), washed twice, and resuspended at the appropriate concentration in 10 mM $MgCl_2$. Inoculum concentrations were determined by optical density at $OD_{600}$ and confirmed by plating serial dilutions on KMB plates. Bacterial suspensions were infiltrated into fully developed tobacco leaves using a 5-mL plastic syringe without a needle (Klement, 1963). The syringe was placed on the abaxial side of the leaf in the laminar area between two lateral veins while the area opposite the site of contact was supported with the other hand; the inoculum was then slowly injected into the leaf intercellular space. Approximately 50 μL of inoculum was infiltrated per panel, forming an infiltrated area of ~20 × 20 mm. For determining bacterial growth in the plant, leaves were inoculated as described above using an inoculum of $10^6$ colony-forming units (cfu)/mL. At each time point, nine 1-$cm^2$ leaf discs were excised from the inoculated tissue of each treatment. The nine discs were divided into three sets of three and macerated in 10 mM $MgCl_2$. The bacterial populations in the leaves were determined by plating serial dilutions on KMB supplemented with 100 mg/L rifampicin. For the *P. s. tabaci* strain expressing *avrPto*, 25 mg/L kanamycin was also added to the media. Tobacco plants were maintained in the greenhouse at 26 to 28°C and 16 hr of light per day.

### DNA Gel Blot Analysis of Transformants

Tobacco genomic DNA was isolated from leaves using the procedure of Dellaporta et al. (1983). Agarose gels were blotted onto Hybond $N^+$ membrane (Amersham), and the filters were hybridized by using a random hexamer $^{32}P$-labeled *Pto* polymerase chain reaction product (1 to 2 × $10^6$ cpm/mL buffer) amplified from the cDNA clone CD186 (Martin et al., 1993b). The filters were washed to 0.5 × SSC (1 × SSC is 0.15 M NaCl, 0.015 M sodium citrate) at 65°C and exposed to x-ray film for 24 hr.

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