Research Note

avrPto* Enhances Growth and Necrosis Caused by *Pseudomonas syringae* pv. *tomato* in Tomato Lines Lacking Either *Pto* or *Prf

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***AvrPto* was introduced into three tomato genotypes with two biotic agents to study its role in compatible interactions. *avrPto* enhanced the capacity of the *Pseudomonas syringae* pv. *tomato* strain T1 to induce necrotic symptoms on tomato plants that lacked either *Pto* or *Prf* genes. The enhanced necrosis correlated with a small increase in bacterial growth. In planta expression of *avrPto* in isolation did not elicit necrosis in the absence of a functional *Prf* gene.**

Many phytopathogenic bacteria carry avirulence genes. Direct or indirect recognition of avirulence gene products by corresponding host-encoded resistance gene products leads to induction of plant defenses (Flor 1956; Keen 1990; Baker et al. 1997). In the absence of one or both corresponding gene(s), the pathogen escapes recognition and is able to replicate to high levels. Recognition of many bacterial avirulence gene products appears to take place within the host cell (reviewed in Bonas and Van den Ackerveken 1997). In such cases, delivery of the avirulence determinant is dependent on *hrp* (for hypersensitive response and pathogenicity) genes, which encode a type III protein secretion apparatus (Alfano and Collmer 1997; Lindgren 1997; Hueck 1998; Anderson et al. 1999; Rossier et al. 1999). Evidence is accumulating that bacterial avirulence genes may contribute to fitness in the absence of host resistance (Kearney and Staskawicz 1990; Swarup et al. 1991, 1992; Lorang et al. 1994; Yang et al. 1994; Ritter and Dangel 1995; Jackson et al. 1999). This provides an explanation for the persistence of genes encoding proteins that can lead to the inhibition of bacterial replication if detected by the host.

Pseudomonas syringae pv. *tomato* (*Pst*) causes bacterial speck disease on tomato, leading to the formation of small, necrotic flecks surrounded by chlorotic haloes on infected

leaves. Strains of *Pst* that express the avirulence gene *avrPto* elicit specific disease resistance on hosts carrying the *Pto* gene. Resistance is manifested as a loss of symptoms at low inoculation levels, or a hypersensitive response (HR) at high bacterial densities. *Pto*-mediated resistance is dependent on a second host gene, *Prf*. Mutations in *Pto* cause a partial loss of *avrPto*-specific resistance, whereas *Prf* mutants are completely susceptible (Salmeron et al. 1994). A current model for signal transduction in this system places *Pto* as the receptor for *AvrPto* (Scofield et al. 1996; Tang et al. 1996), with *Prf* coincident or downstream of the recognition event (Rathjen et al. 1999).

avrPto, like certain other avirulence genes, may confer the ability to modify host defense responses. Genomic expression of *avrPto* from the heterologous pathogen *Potato virus X* (PVX) conferred avirulence on *Pto*-expressing plants (Tobias et al. 1999). In contrast, infection of tomato plants lacking either *Pto* or *Prf* genes resulted in enhanced necrotic symptoms relative to infections with wild-type PVX (Tobias et al. 1999). Therefore, we investigated whether the presence of *avrPto* could modify the response of susceptible tomato hosts to *Pst* infection.

Resistant (Rio Grande 76R [*Pto/Pto Prf/Prf*]) or near-isogenic susceptible (Rio Grande 76S [*pto/pto Prf/Prf*]) tomato lines were vacuum infiltrated with *Pst* strains carrying an empty vector (pDSK519) or pDSK519 carrying *avrPto* (pPtE6; Ronald et al. 1992). *Pst* T1 carrying empty vector caused typical disease symptoms on both genotypes (Table 1 and Fig. 1). Leaves had multiple necrotic flecks indicative of bacterial speck disease, but otherwise remained green. 76R plants showed few or no disease symptoms in response to *Pst* T1 expressing *avrPto*, due to the resistance response mediated by *Pto*. 76S plants infected with *Pst* T1 (*avrPto*) exhibited disease symptoms 4 days post inoculation (dpi), and leaves began to dehydrate after 6 dpi. This *avrPto*-dependent effect was examined in completely susceptible plants lacking a functional *Prf* gene (Rio Grande 76R *prf*-3 [*Pto/Pto prf3/prf3*]). 76R *prf*-3 plants infected with *Pst* T1 developed bacterial speck symptoms similar to 76R and 76S genotypes.

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However, infection of 76R prf-3 plants with *Pst* T1 (*avrPto*) resulted in a more rapid and exaggerated response; symptoms began to develop 4 dpi and leaves became black and brittle by 6 dpi (Fig. 1). The increased necrosis in 76S plants due to the presence of *avrPto* is referred to as “enhanced necrosis.” The *avrPto*-dependent increased necrosis in 76R prf-3 relative to 76S is referred to as “severe necrosis.” The same severe necrotic phenotype was also observed in *Pst* T1(*avrPto*) infections of five other 76R tomato lines with mutations in the *Prf* gene: prf-4, prf-2, prf-16, prf-19 (Salmeron et al. 1994), and the *Prf* sense-suppressed line, pSOR-2 (Oldroyd and Staskawicz 1998). Thus, the presence of *avrPto* appeared to modify the host response to *Pst* T1 infection, similar to the PVX data (Tobias et al. 1999).

Bacterial growth was measured to determine whether the enhanced necrosis associated with *Pst* T1(*avrPto*) infections in susceptible genotypes correlated with increased pathogen replication. 76S and 76R prf-3 plants were vacuum infiltrated with 1×10^3 CFU per ml of *Pst* T1 carrying empty vector or *avrPto*. A small increase in bacterial growth was associated with presence of the *avrPto* gene. *Pst* T1 (*avrPto*) consistently replicated to higher levels than strains lacking *avrPto* at 4 dpi (Fig. 2A). Parallel differences were observed when plants were vacuum infiltrated with *Pst* T1 at 1×10^5 CFU per ml (Fig. 2B). The differences in growth were statistically significant ($F = 18.57$, $P < 0.001$) and observed in multiple independent experiments. Bacterial growth was not significantly influenced by plant genotype and the differences in severity of necrosis between 76S and 76R prf-3 did not correlate with differences in bacterial replication.

Expression of *avrPto* from *Pst* T1 or PVX caused enhanced necrosis in susceptible tomato hosts. To examine whether induction of necrosis was a primary consequence of AvrPto within the plant cell, we expressed *avrPto* in planta with *Agrobacterium*-mediated transient expression. Tomato plants were pressure infiltrated with suspensions of the *A. tumefaciens* strain C58C1 carrying either *Pto* alone, *avrPto* alone, or both *avrPto* and *Pto* together on the same T-DNA (Fig. 3) (Schofield et al. 1996; Rathjen et al. 1999). Expression of wild-type *Pto* did not elicit an HR in any of the three tomato lines. Co-expression of *avrPto* and *Pto* from the same T-DNA elicited an HR in leaves of 76R and 76S, but not in leaves of 76R prf-3. Expression of *avrPto* alone in 76R leaf tissue elicited an HR due to *Pto*-mediated recognition. Expression of *avrPto* in 76S tissue caused spotty, nonconfluent tissue collapse within

the site of infiltration. In contrast, no effect of *avrPto* expression was observed in leaves of 76R prf-3. Therefore, expression of *avrPto* did not induce necrosis in the absence of a functional *Prf* gene.

Our data show that expression of *avrPto* from *Pst* T1 was associated with an increase in necrotic disease symptoms in susceptible tomato lines. The enhanced necrosis was associated with a slight but significant increase in bacterial replication. However, *avrPto* did not elicit necrosis when expressed in isolation in the absence of a functional *Prf* gene. Together, these data show that *avrPto* is necessary but not sufficient for induction of the pathogen-associated necrosis observed in compatible interactions. *avrPto* appears to modify the interaction between plant and pathogen leading to an altered host reaction to infection. This may be indicative of suppression of a general defense response, given that the effect was seen with unrelated pathogens. However, the data presented here cannot discriminate whether AvrPto caused its effect within the bacterial or plant cells. Also, we are unable to determine the importance of the *Pto* gene product in influencing the necrotic phenotype in 76R prf-3, because the extremely tight physical linkage of *Pto* and *Prf* precludes generation and analyses of a *pto/pto prf/prf* genotype.

We observed an apparent minor recognition event of *avrPto* when expressed in wild-type susceptible tomato (76S), as evidenced by development of a limited HR. This observation has been recorded previously (Gopalan et al. 1996), and is consistent with mutational studies in which mutants in the *Pto* gene were partially resistant to *Pst* (*avrPto*) infection, but mutants in *Prf* were fully susceptible (Salmeron et al. 1994). It is possible that one of the five *Pto* homologs encoded at the locus mediates this recognition, although no interaction between AvrPto and any of the homologs was detected with yeast two-hybrid analysis (J. Chang, B. Staskawicz, and R. Michelmore, *unpublished*). It is also possible that *Prf* itself can act as receptor for AvrPto; however, an interaction between AvrPto and *Prf* was not necessary for induction of an HR induced by expression of a constitutive gain-of-function mutant of *Pto* (Rathjen et al. 1999). The implication of a minor *avrPto* recognition determinant at the *Pto* locus is interesting because *Pst* DC3000 encodes a second avirulence determinant that is also recognized by a *Prf*-dependent process (Ronald et al. 1992; Salmeron et al. 1994). Therefore, the *Pto* locus appears to determine multiple molecular interactions with *Pst*.

Table 1. Summary of plant responses to AvrPto delivered by strains of *Pseudomonas syringae*, *Agrobacterium tumefaciens*, or Potato virus X (PVX)

Biological agent	Tomato genotype		
	Rio Grande 76R	Rio Grande 76S	Rio Grande 76R prf-3
<i>P. syringae</i> pv. <i>tomato</i> T1 carrying vector	Bacterial speck disease 4 dpi onward ^a	Bacterial speck disease 4 dpi onward	Bacterial speck disease 4 dpi onward
<i>P. syringae</i> pv. <i>tomato</i> T1 expressing <i>avrPto</i>	No or very few symptoms 6 dpi	Disease symptoms 4 dpi. Necrosis. Leaves dry but not black as in prf-3 plants 6 dpi	Disease symptoms 4 dpi. Severe necrosis. Leaves dry and black 6 dpi
<i>A. tumefaciens</i> carrying vector	No phenotype	No phenotype	No phenotype
<i>A. tumefaciens</i> carrying <i>avrPto</i>	Hypersensitive response ^b	Non-confluent HR	No phenotype
PVX	Systemic chlorosis	Systemic chlorosis	Systemic chlorosis
PVX:: <i>avrPto</i> (Tobias et al. 1999)	No macroscopic phenotype	Varying degrees of tissue necrosis, from individual leaves to entire plant	Necrosis of entire plant

^a dpi, days post inoculation.

^b Hypersensitive response.

Fig. 1. *Pseudomonas syringae* pv. *tomato* T1 expressing *avrPto* causes enhanced necrosis in plants lacking functional *Pto* or *Prf* genes. Depicted are responses by 76S and 76R *prf-3* to vacuum infiltration of 1×10^5 CFU per ml of *P. syringae* pv. *tomato* expressing (A and C) *avrPto* or (B and D) pDSK519. Plants were the same age and approximate size at time of infiltration. Leaf pairs (A and C, and B and D) were of similar size prior to infiltration and were taken from comparable positions on their respective plants. Photographs were taken 8 days post inoculation.

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Fig. 2. Multiplication of *Pseudomonas syringae* pv. *tomato* T1 in 76S and 76R *prf-3*. *P. syringae* pv. *tomato* T1 carrying *avrPto* or pDSK519 were vacuum infiltrated at (A) 1×10^3 CFU per ml or (B) 1×10^5 CFU per ml in 76S or 76R *prf-3*. Leaf samples were taken daily for 5 days. Data points represent the mean of three leaf samples plus or minus standard error.

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Fig. 3. *Agrobacterium*-mediated transient expression of *Pto*, *avrPto*, and *Pto* and *avrPto* together in tomato genotypes 76R, 76S, or 76R prf-3. *A. tumefaciens* C58C1 (pCH30) carrying each of the constructs was pressure infiltrated into leaves of 3- to 4-week-old tomato plants. Black lines delineate boundaries of the *A. tumefaciens* suspension following infiltration. Photographs were taken 2.5 days post inoculation. Results are representative of at least three separate infiltrations.

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