

Resistance to Turnip Crinkle Virus in *Arabidopsis* Is Regulated by Two Host Genes and Is Salicylic Acid Dependent but *NPR1*, Ethylene, and Jasmonate Independent

Pradeep Kachroo,^{a,b} Keiko Yoshioka,^{a,b} Jyoti Shah,^{a,b,1} Hugo K. Dooner,^{a,c} and Daniel F. Klessig^{a,b,2}

^a Waksman Institute, Rutgers, State University of New Jersey, Piscataway, New Jersey 08854-8020

^b Department of Molecular Biology and Biochemistry, Rutgers, State University of New Jersey, Piscataway, New Jersey 08854-8020

^c Department of Plant Science, Rutgers, State University of New Jersey, New Brunswick, New Jersey 08903-0231

Inoculation of turnip crinkle virus (TCV) on the resistant *Arabidopsis* ecotype Dijon (Di-17) results in the development of a hypersensitive response (HR) on the inoculated leaves. To assess the role of the recently cloned *HRT* gene in conferring resistance, we monitored both HR and resistance (lack of viral spread to systemic tissues) in the progeny of a cross between resistant Di-17 and susceptible Columbia plants. As expected, HR development segregated as a dominant trait that corresponded with the presence of *HRT*. However, all of the F₁ plants and three-fourths of HR⁺ F₂ plants were susceptible to the virus. These results suggest the presence of a second gene, termed *RRT*, that regulates resistance to TCV. The allele present in Di-17 appears to be recessive to the allele or alleles present in TCV-susceptible ecotypes. We also demonstrate that HR formation and TCV resistance are dependent on salicylic acid but not on ethylene or jasmonic acid. Furthermore, these phenomena are unaffected by mutations in *NPR1*. Thus, TCV resistance requires a yet undefined salicylic acid-dependent, *NPR1*-independent signaling pathway.

INTRODUCTION

Plants resisting pathogen attack frequently activate a variety of defense responses that are initiated by the direct or indirect interaction between the products of a plant-encoded resistance (*R*) gene and its corresponding pathogen-encoded avirulence (*Avr*) gene. A majority of such interactions also result in the induction of host cell death at the site of pathogen infection, a phenomenon known as the hypersensitive response (HR). Subsequent to the HR, the uninoculated tissues usually develop a long-lasting, enhanced resistance to further attacks by the same or even unrelated microbial pathogens; this is referred to as systemic acquired resistance (SAR) (Ryals et al., 1996; Durner et al., 1997; Dempsey et al., 1999).

Although the HR is one of the earliest manifestations of disease resistance, it remains unclear whether this phenomenon is a prerequisite for gene-for-gene-mediated disease

resistance or whether it simply results from the activation of multiple defense signaling pathways. For example, the HR does not develop in potato during *Rx*-mediated resistance against potato virus X (Köhm et al., 1993) or in the *Arabidopsis dnd1* mutant after pathogen infection (Yu et al., 1998). Similarly, in barley, the HR is dispensable in *Mlg*-specified resistance to the powdery mildew fungus (Koga et al., 1990; Schiffer et al., 1997). Several studies on tobacco infected with tobacco mosaic virus (TMV) have also indicated that localization of the virus is dependent on accumulation of salicylic acid (SA) and not cell death (Bi et al., 1995; Mittler et al., 1996; Ryals et al., 1996; Mur et al., 1997). In contrast, an HR is required for *Mla*-specified resistance to powdery mildew in barley (Koga et al., 1990; Schiffer et al., 1997). Therefore, the relationship between HR and disease resistance (cause or consequence) most probably depends on the specifics of the interaction between the resistance and avirulence gene products and the downstream components activated as a result of their interaction.

Many studies have demonstrated that SA is an important component of the signal transduction pathway leading to disease resistance (Durner et al., 1997; Dempsey et al., 1999). For example, plants unable to accumulate SA because of the expression of a bacterial *nahG* gene encoding salicylate

¹ Current address: Division of Biology, Kansas State University, 303 Ackert Hall, Manhattan, KS 66506.

² To whom correspondence should be addressed. E-mail klessig@mbcl.rutgers.edu; fax 732-445-5735.

hydroxylase fail to develop SAR and exhibit heightened susceptibility to pathogen infection (Gaffney et al., 1993; Delaney et al., 1994). In addition to SA, ethylene and jasmonic acid (JA) serve as important signals for the induction of various defense responses. JA and ethylene have been implicated in (1) resistance to several fungi (Knoester et al., 1998; Thomma et al., 1998; Vijayan et al., 1998), (2) induced systemic resistance triggered by *Pseudomonas fluorescens* (Pieterse et al., 1998; Pieterse and Van Loon, 1999), (3) activation of genes encoding thionin and defensin (Epple et al., 1995; Penninckx et al., 1996, 1998), and (4) activation of enzymes involved in phytoalexin synthesis (Boller et al., 1983; Ecker and Davis, 1987; Mauch and Staehelin, 1989). Interestingly, ethylene and JA work in conjunction with SA to signal some but not all defense responses (Dong, 1998).

Genetic analysis of the SA-regulated pathway leading to disease resistance has revealed that a key signaling component is encoded by the *NPR1/NIM1* gene (Cao et al., 1994, 1997; Delaney et al., 1995; Glazebrook et al., 1996; Ryals et al., 1997; Shah et al., 1997). Arabidopsis mutants lacking a functional *NPR1/NIM1* gene are unable to express the pathogenesis-related (*PR*) genes in response to SA or its functional analogs 2,6-dichloroisonicotinic acid and benzothiadiazole. In addition, *npr1/nim1* plants show enhanced susceptibility to both bacterial and fungal pathogens (Cao et al., 1994; Delaney et al., 1995; Glazebrook et al., 1996; Shah et al., 1997). Conversely, overexpression of *NPR1* confers resistance against both bacterial and fungal pathogens in a dosage-dependent manner (Cao et al., 1998). Whereas *NPR1* plays a critical role in resistance to various bacterial and fungal pathogens, its involvement in viral resistance is not known.

In Arabidopsis, resistance to most viral pathogens does not involve an HR (Ishikawa et al., 1991; Leisner et al., 1993; Lee et al., 1994; Callaway et al., 1996). However, inoculation of turnip crinkle virus (TCV) (Morris and Carrington, 1988) on plants from the resistant ecotype Dijon (Di-0 or Di-17) results in both an HR and the induction of *PR* gene expression (Simon et al., 1992; Dempsey et al., 1993, 1997; Uknes et al., 1993). In contrast, TCV-susceptible ecotypes, including Columbia (Col-0), fail to mount an HR, exhibit delayed and weak *PR* gene expression, and develop systemic disease symptoms (Li and Simon, 1990; Dempsey et al., 1993). Genetic analyses revealed that HR development is conferred by a single dominant gene termed *HRT* (for HR to TCV) (Dempsey et al., 1997). *HRT* also appears to be required for resistance to TCV infection; all of the HR⁻ progeny from crosses between resistant and susceptible ecotypes developed systemic disease symptoms. However, *HRT* alone may not be sufficient for complete resistance because many of the HR⁺ progeny also succumbed to infection. In this study, we show that TCV resistance is influenced by a second locus, named *RRT* (for regulates resistance to TCV). Furthermore, we demonstrate that the HR and resistance are dependent on SA but independent of *NPR1*-, ethylene-, and JA-mediated defense signaling.

RESULTS

Defense Gene Expression during the Resistance Response

Several families of *PR* genes have been shown to be activated in Di-17 plants that resist TCV infection (Dempsey et al., 1993; Uknes et al., 1993). To determine whether other known defense genes are induced during this resistance response, we extracted total RNA from the TCV-inoculated and mock-inoculated leaves of Di-17 and Col-0 plants at 1 to 3 days postinoculation (DPI). The accumulation of gene transcripts for *PR-1*, phenylalanine ammonia lyase (*PAL*) (Wanner et al., 1995), glutathione *S*-transferase (*GST1* and *GST6*) (Chen et al., 1996; Yang et al., 1999), defensin (*PDF1.2*) (Penninckx et al., 1996), thionin (*THI2.1*) (Epple et al., 1995), ascorbate peroxidase (*APX1* and *APX2*) (Kubo et al., 1993; Karpinski et al., 1997), lipoxygenase (*LOX2*) (Bell and Mullet, 1993), *NPR1* (Cao et al., 1997), 1-aminocyclopropane-1-carboxylic acid (*ACC*) synthase (Van der Straeten et al., 1992), and alternative oxidase (*AOX*) (Kumar and Soll, 1992) were then analyzed by reverse transcription-polymerase chain reaction (RT-PCR) and RNA gel blot analysis (Figure 1 and data not shown). Whereas the transcripts for *ACC* synthase and *AOX* accumulated to very low amounts (data not shown), transcripts for *PR-1* and *GST1* accumulated to high amounts in the TCV-inoculated leaves of Di-17 plants (Figure 1). Expression of the other genes was not induced in these leaves (Figure 1 and data not shown). In contrast, the TCV-inoculated leaves of Col-0 plants showed only basal-level expression for all of the defense genes assayed (Figure 1).

Genetic Characterization of Resistance against TCV

We previously showed that the HR in TCV-infected plants is dependent on the dominant *HRT* gene (Dempsey et al., 1997). However, because many progeny from a Di-17 × Col-0 cross are HR⁺ but TCV susceptible, this gene may not be sufficient to confer complete resistance. To further characterize the role of *HRT* in TCV-infected plants, we crossed Di-17 and Col-0 plants. All of the F₁ progeny developed an HR on the TCV-inoculated leaves (Table 1) and accumulated increased amounts of *PR-1* and *GST1* transcripts (Figure 1). Nonetheless, these F₁ plants allowed systemic spread of the virus (Figure 1) and developed disease symptoms (crinkled leaves and drooping bolts; Table 1). One explanation for the TCV-susceptible phenotype of these F₁ plants might be that resistance requires homozygosity for the *HRT* allele. Alternatively, a second recessive gene, in addition to *HRT*, might regulate resistance.

To determine whether resistance depends on *HRT* dosage or the presence of a second gene, we analyzed F₂ progeny from the Di-17 × Col-0 cross. Of the 458 F₂ plants, 373 were susceptible and 85 were resistant. The ratio between

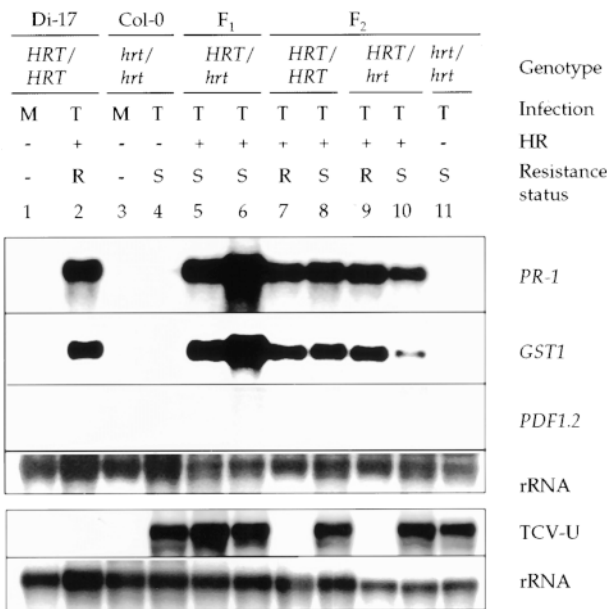


Figure 1. *PR-1*, *GST1*, and *PDF1.2* Expression and Systemic Spread of TCV in Di-17 × Col-0 F₁ and F₂ Plants.

Expression of *PR-1*, *GST1*, and *PDF1.2* in the inoculated leaves of TCV (T)- and mock (M)-infected Di-17 and Col-0 plants and the F₁ and F₂ progeny obtained from a cross between them. The inoculated leaves were monitored for the presence (+) or absence (-) of the HR, and the RNA was extracted from the inoculated leaves at 4 DPI. The replication and spread of the virus were analyzed by extracting RNA from the uninoculated (TCV-U) bolt tissue at 10 DPI. Two F₁ and five F₂ plants were analyzed. The genotype of the F₂ plants at the *HRT* locus was determined by CAPS analysis; both homozygous and heterozygous plants were analyzed for the expression of defense genes in inoculated leaves, the presence of TCV RNA in the uninoculated bolt tissue, and the appearance (S) or absence (R) of disease symptoms. The blot (1 to 11) was sequentially probed for the indicated genes (*PR-1*, *GST1*, and *PDF1.2*). RNA extracted from the systemic tissues was run on a separate gel; rRNA was used as an internal control for gel loading and transfer for both blots.

susceptible and resistant plants fits very closely the 13:3 segregation expected if resistance is due to at least one dose of *HRT* and homozygosity for a recessive gene at an unlinked locus ($\chi^2 = 0.01$; $0.95 > P > 0.8$), a gene we have named *RRT*. In contrast, if resistance were dependent on two doses of *HRT*, ~33% of the HR⁺ F₂ plants (122) should have been resistant. Because the data obtained do not fit the Mendelian ratio of 2:1 ($\chi^2 = 16.4$; $P < 0.01$), we can reject this possibility. As expected, the HR segregated as a dominant trait (Table 1; 365 HR⁺/93 HR⁻ plants; $\chi^2 = 5.08$; $0.05 > P > 0.01$), although the number of HR⁺ plants (365) was somewhat higher than expected (344). This greater number of HR⁺ plants could be the result of differential transmission at the *HRT* locus, and indeed, genotype analysis showed that this was the case (see below).

To provide additional support for our hypothesis that both *HRT* and *RRT* regulate resistance to TCV, we monitored the *HRT* genotype, the ability to form an HR, and viral resistance in a population of 355 F₂ plants derived from a cross between Di-17 and Col-0 plants (Table 2). Using a cleaved amplified polymorphic sequence (CAPS) marker that can differentiate between the *HRT* allele of Di-17 and the *hrt* allele of Col-0 (Cooley et al., 2000), we identified 119 plants as *HRT/HRT*, 164 as *HRT/hrt*, and 72 as *hrt/hrt*. All of the *HRT*⁻ plants developed HR at 3 DPI, whereas none of the *hrt/hrt* plants showed any visible lesions, confirming the requirement of *HRT* for the HR. As was previously observed, the number of HR⁺ plants (283) was somewhat more than what was expected (267). Moreover, substantially more of these plants were homozygous for the *HRT* allele (119) than would be predicted (89) by a Mendelian ratio of 1:2:1 (Table 2). Thus, recovery of the dominant *HRT* allele (57%) in the F₂ is significantly higher than that of the recessive allele (43%) ($\chi^2 = 12.4$; $P < 0.01$). Similar results were obtained when selfing other F₁ plants, suggesting preferential transmission of *HRT* by one or both gametophytes in this specific F₁ hybrid.

Further analysis of these F₂ plants confirmed that *HRT* is also required for resistance. All 72 of the *hrt/hrt* plants not only failed to develop an HR but also were susceptible to TCV infection. If resistance were not dependent on *HRT* but required only *rrt*, then ~18 *hrt/hrt* plants should have been resistant. In addition, resistance does not correlate with the presence of two doses of the *HRT* allele; 42 of the 119 *HRT/HRT* plants and 19 of 164 *HRT/hrt* plants were TCV resistant (Table 2). Rather, the data are more consistent with the numbers predicted if a second locus, in addition to *HRT*, is required for resistance. In this scenario, 25% of the *HRT/HRT* plants (30) and 25% of the *HRT/hrt* plants (41) should be resistant. The number of *HRT/HRT*-resistant plants (42) was somewhat more than expected, and the number of *HRT/hrt*-resistant plants (19) was only half of that expected. A likely explanation for the discrepancy between the observed and expected numbers is that the increased dosage of *HRT* enhances the durability of resistance or reduces the extent to which environmental factors affect the expressivity of *HRT*. Further supporting this possibility is the observation that increased expression of *HRT* in transgenic Col-0 plants leads to enhanced resistance to TCV (Cooley et al., 2000).

To determine whether *HRT* dosage has any effect on the extent of viral spread and defense gene expression, we monitored these phenomena in a subset of the Di-17 × Col-0 F₂ progeny. All of the plants exhibiting disease symptoms were found to contain viral RNA in the uninoculated tissue. These plants accumulated similar amounts of viral RNA, regardless of whether they contained zero, one, or two doses of the *HRT* allele (Figure 1). In contrast, *PR-1* and *GST1* expression in the TCV-inoculated leaves occurred only in F₂ progeny containing at least one dose of the *HRT* allele and independent of their resistance phenotype. Comparable expressions of these genes were generally detected in homozygous and

Table 1. Segregation of the HR and Resistance in a Cross between Di-17 and Col-0 Plants

Cross ^a	Generation	Total No. of Plants	Phenotype of Plants				χ^2 ^b	P
			HR ⁺ /S ^c	HR ⁺ /R ^d	HR ⁻ /S	HR ⁻ /R		
Di-17 × Col-0	F ₁	6	6	0	0	0	0.01	>0.8
	F ₂	458	280	85	93	0		

^a The pollen-accepting plant is indicated first and the pollen donor second.

^b Calculation based on a 13:3 segregation.

^c Susceptible, disease symptoms include crinkling of leaves and drooping of the bolt.

^d Resistant, no disease symptoms.

heterozygous plants, suggesting that these events are not sufficient to confer resistance (Figure 1).

Role of SA in HR Development and Resistance to TCV

We have previously shown that in *Arabidopsis*, increases in SA and its glucoside (SAG) correlate with resistance to TCV (Dempsey et al., 1997). To further explore the role of SA in TCV resistance, we first tested whether exogenously applied SA confers resistance to plants from the susceptible ecotypes Col-0 and Nössen (Nö). Two days after treatment with SA, plants were infected with TCV and then monitored for development of an HR, the appearance of disease symptoms, and the presence of TCV in uninoculated tissue. At 3 DPI, no macroscopic HR was evident on the TCV-inoculated leaves of SA-treated Col-0 plants. However, by 10 DPI, disease symptoms were evident (data not shown), and viral RNA had accumulated to very high amounts in the systemic leaves (Figure 2). No differences in the amounts of viral RNA were observed between the SA-treated and water-treated control Col-0 plants. To ensure that the SA treatment was effective in inducing defense signaling, we monitored *PR-1* expression in the inoculated and systemic tissues at 10 DPI. SA treatment was observed to induce *PR-1* expression in these plants (Figure 2); however, that was insufficient to confer resistance to TCV. Similar results were obtained with Nö plants (data not shown).

We next determined whether constitutively increased amounts of endogenous SA would confer TCV resistance.

Several *Arabidopsis* mutants have been isolated that exhibit enhanced resistance to bacterial and fungal pathogens, constitutively express *PR* genes, and have increased SA/SAG (Durner et al., 1997; Yang et al., 1997). Of these, the *cpr5* (Bowling et al., 1997), *cep* (Silva et al., 1999), *ssi1* (Shah et al., 1999), *ssi2* (J. Shah, P. Kachroo, and D.F. Klessig, unpublished results), and *cpr22* (K. Yoshioka, F. Tsui, P. Kachroo, S.B. Sharma, and D.F. Klessig, unpublished results) mutants were analyzed for resistance to TCV. All of these mutants are derived from the TCV-susceptible Col-0, Nö, or Wassilewskija ecotypes. When infected, all exhibited severe disease symptoms and accumulated TCV RNA in the uninoculated tissues in amounts similar to those observed in the respective TCV-infected wild-type parents (data not shown). Thus, increased endogenous SA/SAG and the constitutive expression of *PR* genes are not sufficient to confer resistance to TCV.

Although the preceding studies demonstrate that increased amounts of SA do not confer resistance to susceptible plants, they do not address whether SA is involved in activating resistance in Di-17 plants. Therefore, we tested the effect of the *nahG*-encoded salicylate-degrading enzyme salicylate hydroxylase on HR development and TCV resistance. Reciprocal crosses were made between Di-17 and NahG transgenic (ecotype Nö) plants. Because both *HRT* and the *nahG* transgene are dominant, the phenotypes were monitored in two F₁ progeny from each cross (Table 3). As a control, HR development and resistance to TCV also were assayed in F₁ progeny from a cross between Di-17 and wild-type Nö plants. All four F₁ plants derived from the Di-17 ×

Table 2. Segregation of the *HRT* Locus and Resistance in a Cross between Di-17 and Col-0 Plants

Cross ^a	Generation	Total No. of Plants	Genotype ^b at the <i>HRT</i> Locus			Resistant ^c Plants		
			<i>HRT/HRT</i>	<i>HRT/hrt</i>	<i>hrt/hrt</i>	<i>HRT/HRT</i>	<i>HRT/hrt</i>	<i>hrt/hrt</i>
Di-17 × Col-0	F ₂	355	119	164	72	42	19	0

^a The pollen-accepting plant is indicated first and the pollen donor second.

^b The genotype at *HRT* was determined by CAPS analysis.

^c Resistant, no disease symptoms.

NahG Nö cross accumulated transcripts for the *nahG* transgene and failed to express the prototypical SA-induced *PR-1* gene after TCV infection (Figure 3 and Table 3). These plants, like the parental NahG Nö transgenic and wild-type Nö plants, failed to develop an HR after TCV infection. In contrast, the parental Di-17 plants and three F₁ plants derived from Di-17 × Nö cross developed an HR after TCV inoculation (Figure 3 and data not shown). Based on these results, increased amounts of SA are required for the HR to TCV. All four NahG-containing F₁ plants also exhibited disease symptoms and allowed viral replication and spread from the inoculated leaves into the uninoculated tissues (Figure 3). However, because the F₁ progeny from the Di-17 × Nö cross were TCV susceptible, the Nö ecotype did not appear to contain the recessive *rrt* allele required for resistance. Thus, analyses of the F₂ progeny were performed so that the importance of SA in resistance could be assessed.

After self-pollination of an F₁ plant, 139 F₂ progeny were analyzed for HR formation and resistance to TCV as well as the presence of the *nahG* transgene (Table 3). Consistent with our hypothesis that SA is required for the activation of defense responses (including the HR) and resistance to TCV, all of the NahG⁺ plants were HR⁻ and all of the HR⁺ progeny were NahG⁻ (Table 3, and Figures 4 and 5). Moreover, all of the NahG⁺ F₂ progeny were susceptible to TCV infection.

To ensure that the HR⁻ susceptible phenotype in at least a portion of the F₂ plants was the result of SA deficiency rather than absence of the *HRT* allele, the genotypes at the *nahG* and *HRT* loci were tested by PCR and CAPS analysis in 101 of the 139 F₂ plants. From this analysis, 70 NahG⁺ plants were identified; 52 of these were either homozygous or heterozygous for the *HRT* allele, and all were susceptible to TCV. Given that *rrt* segregates as an independent locus, ~13 of these 70 plants should have been *rrt/rrt* and resistant to TCV if SA were not required. Of the 31 NahG⁻ plants, all contained at least one copy of the *HRT* allele and developed an HR after TCV infection. In addition, 16 of these 31 plants were resistant to TCV. This number of resistant plants is more than expected (eight). However, that result would be explained if the NahG transgene is loosely linked to the *RRT* allele in the Nö parents; selecting for NahG⁻ progeny would thereby increase the number of individuals carrying the *rrt* allele associated with resistance. Taken together, these results indicate that both SA and *HRT* are required but not sufficient for resistance to TCV.

Role of *NPR1* in the HR and Resistance to TCV

NPR1 plays an essential role in the SA-mediated signal transduction pathway that leads to the activation of *PR* genes (Cao et al., 1994; Delaney et al., 1995; Glazebrook et al., 1996; Shah et al., 1997). Because SA appears to be required for HR development and resistance, we asked whether *NPR1* plays a role in signaling these phenomena by crossing Di-17 with *npr1-1* (Col-0 ecotype; Cao et al., 1994)

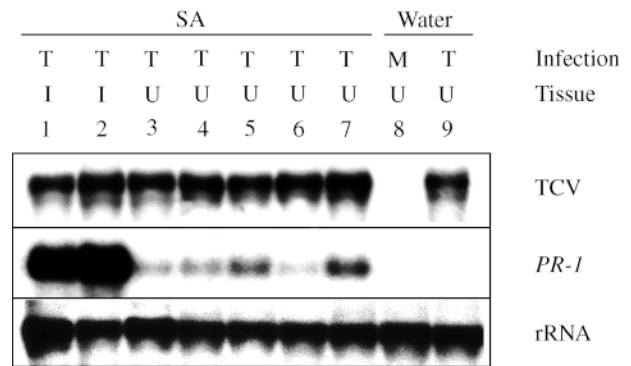


Figure 2. *PR-1* Expression and Systemic Spread of TCV in SA-Treated Plants.

Three-week-old Col-0 plants were treated with SA (500 μM) or water and then TCV (T)- or mock (M)-inoculated 2 days after treatment. The expression of *PR-1* and the accumulation of viral genome were analyzed in both inoculated (I) and uninoculated (U) tissues at 10 DPI. The blot (1 to 9) was sequentially probed for TCV, *PR-1*, and rRNA (the rRNA served as an internal control for gel loading and transfer).

or *npr1-5* (Nö ecotype; Shah et al., 1997) plants. Both of these *npr1* alleles are recessive compared with the wild-type *NPR1* allele. As expected, the two F₁ plants derived from the Di-17 × *npr1-1* cross exhibited an HR after TCV infection but were still susceptible to TCV (Table 4). Using CAPS analysis, we identified F₂ progeny homozygous for *npr1-1* or *npr1-5*. These plants were subsequently monitored for their ability to develop an HR and resist TCV infection. Of 13 *npr1-1/npr1-1*, *HRTI*⁻ segregants, all exhibited an HR and two were TCV resistant (Table 4). Likewise, of 31 *npr1-5/npr1-5*, *HRTI*⁻ F₂ segregants, all were HR⁺ (Figure 6) and nine were TCV resistant (Table 4). Resistance in these nine plants was confirmed by RNA gel blot analysis; no TCV genomic RNA was detected in the uninoculated tissues (data not shown). Because the HR was not blocked by the presence of the *npr1-1* or *npr1-5* alleles and because the number of TCV-resistant progeny expected (based on digenic segregation of *HRT* and *rrt*) was unaffected in *npr1* homozygous plants, *NPR1* is not required for the HR or resistance to TCV, even though SA is. This surprising result argues that resistance to TCV requires a yet to be characterized SA-dependent, *NPR1*-independent signaling pathway.

Because activation of *PR-1* expression in Arabidopsis plants that resist TCV infection correlates with increases in SA contents (Figure 1; Uknes et al., 1993; Dempsey et al., 1997), we tested whether this phenomenon depended on *NPR1*. RNA gel blot analysis of *npr1-5/npr1-5* and *npr1-1/npr1-1* F₂ plants that were homozygous or heterozygous for *HRT* indicated that *PR-1* expression was activated in the inoculated leaves (Figure 7). Although the amount of expression in these leaves was substantial, it was not as strong as

Table 3. Segregation of the HR and Resistance in a Cross between Di-17 and a NahG Transgenic Plant

Cross ^a	Generation	Total No. of Plants	NahG ⁺ /HR ⁺	NahG ⁺ /HR ⁻	NahG ⁻ /HR ⁺	NahG ⁻ /HR ⁻
NahG Nö × Di-17	F ₁	2	0	2 S ^b	0	0
Di-17 × NahG Nö	F ₁	2	0	2 S	0	0
	F ₂	139	0	92 S	21 R ^c ; 23 S	3 S

^aThe pollen-accepting plant is indicated first and the pollen donor second.

^bS, susceptible; disease symptoms include crinkling of leaves and drooping of the bolt.

^cR, resistant; no disease symptoms.

that detected in the inoculated leaves of *NPR1/NPR1* F₂ plants or in the Di-17 controls. Thus, *PR-1* expression appears to be regulated by both *NPR1*-dependent and -independent pathways in TCV-inoculated resistant plants. A similar conclusion has been drawn from studies with the *npr1* mutants and bacterial pathogens (Glazebrook et al., 1996; Shah et al., 1997).

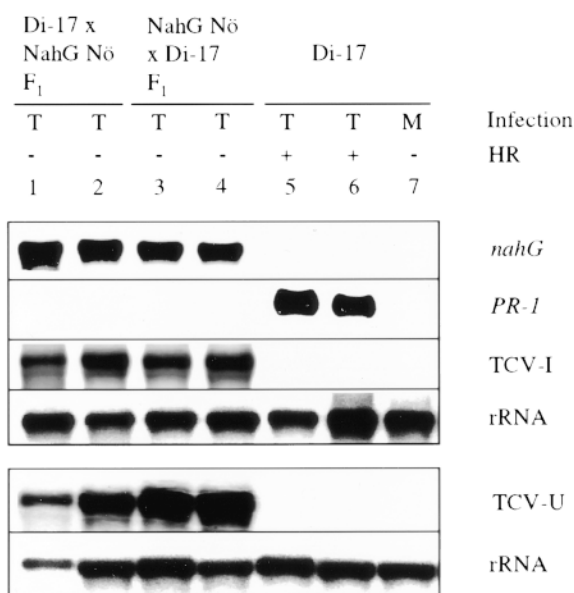


Figure 3. *PR-1* Expression and Systemic Spread of TCV in Di-17 × *nahG* F₁ Plants.

Two F₁ plants each from a cross of Di-17 and NahG Nö and its reciprocal cross were inoculated with TCV and monitored for the presence (+) or absence (-) of the HR. TCV (T)- or mock (M)-inoculated Di-17 parental plants were used as controls. Expression of the *PR-1* gene in the inoculated leaves was analyzed at 4 DPI. The systemic spread and replication of the virus (TCV-U) were determined by analyzing RNA extracted from the uninoculated bolt tissues at 10 DPI. The blot (1 to 7) was sequentially probed for *PR-1*, the *nahG* transgene, and TCV genome. RNA extracted from the systemic tissues was run on a separate gel. rRNA was used as an internal control for gel loading and transfer for both blots.

Role of Ethylene and JA in Signaling HR Development and Resistance to TCV

The role of ethylene and JA signaling in HR development and resistance to TCV was determined by using Arabidopsis mutants insensitive to either of these defense signaling molecules. To analyze the role of ethylene, Di-17 plants were crossed with the dominant ethylene-insensitive *etr1-1* (ecotype Col-0) mutant (Chang et al., 1993). All of the F₁ progeny derived from this cross exhibited an HR but were susceptible to TCV (Table 4). Because the *etr1-1* mutation is dominant, this result suggests that ethylene signaling is not required for the HR to TCV. To determine the effect of *etr1-1* on resistance, 58 *HRT1*- F₂ progeny containing at least one dose of the dominant *etr1-1* allele were identified by CAPS analysis and then infected with TCV. All of these plants exhibited an HR (Figure 6; $\chi^2_{\text{for } 3:1} = 0.44$; $P = 0.5$), and 14 of these were resistant to TCV (Table 4; $\chi^2_{\text{for } 9:3:4} = 0.462$; $0.95 > P > 0.8$). Resistance in these plants was confirmed by RNA gel blot analysis, which failed to detect TCV genomic RNA in the uninoculated tissues (Figure 8). Because the number of HR⁺ TCV-resistant plants conformed to previously observed digenic segregation ratios, despite the presence of *etr1-1*, we conclude that this mutation and therefore ethylene signaling had no effect on either HR formation or TCV resistance.

The involvement of JA in the activation of defense responses against TCV was assessed by crossing the jasmonate-insensitive *coi1-1* mutant (ecotype Col-0) (Xie et al., 1998) with a Di-17 plant. The F₁ progeny all developed an HR but were susceptible to TCV. To determine the effect of the recessive *coi1-1* mutation on HR and resistance, 27 F₂ plants homozygous for the *coi1-1* allele and containing at least one copy of the *HRT* allele were identified by CAPS analysis. All of these plants developed an HR (Figure 6; $\chi^2_{\text{for } 3:1} = 0.28$; $0.8 > P > 0.5$), and six were resistant to TCV (Table 4; $\chi^2_{\text{for } 9:3:4} = 0.41$; $0.95 > P > 0.8$). The above ratios conform to what would be expected if the *coi1-1* mutation had no effect on either the HR or resistance. Hence, JA, like ethylene, is not required for development of the HR or resistance to TCV.

In the previous section, we showed that although TCV-induced *PR-1* expression is SA dependent, it can be activated

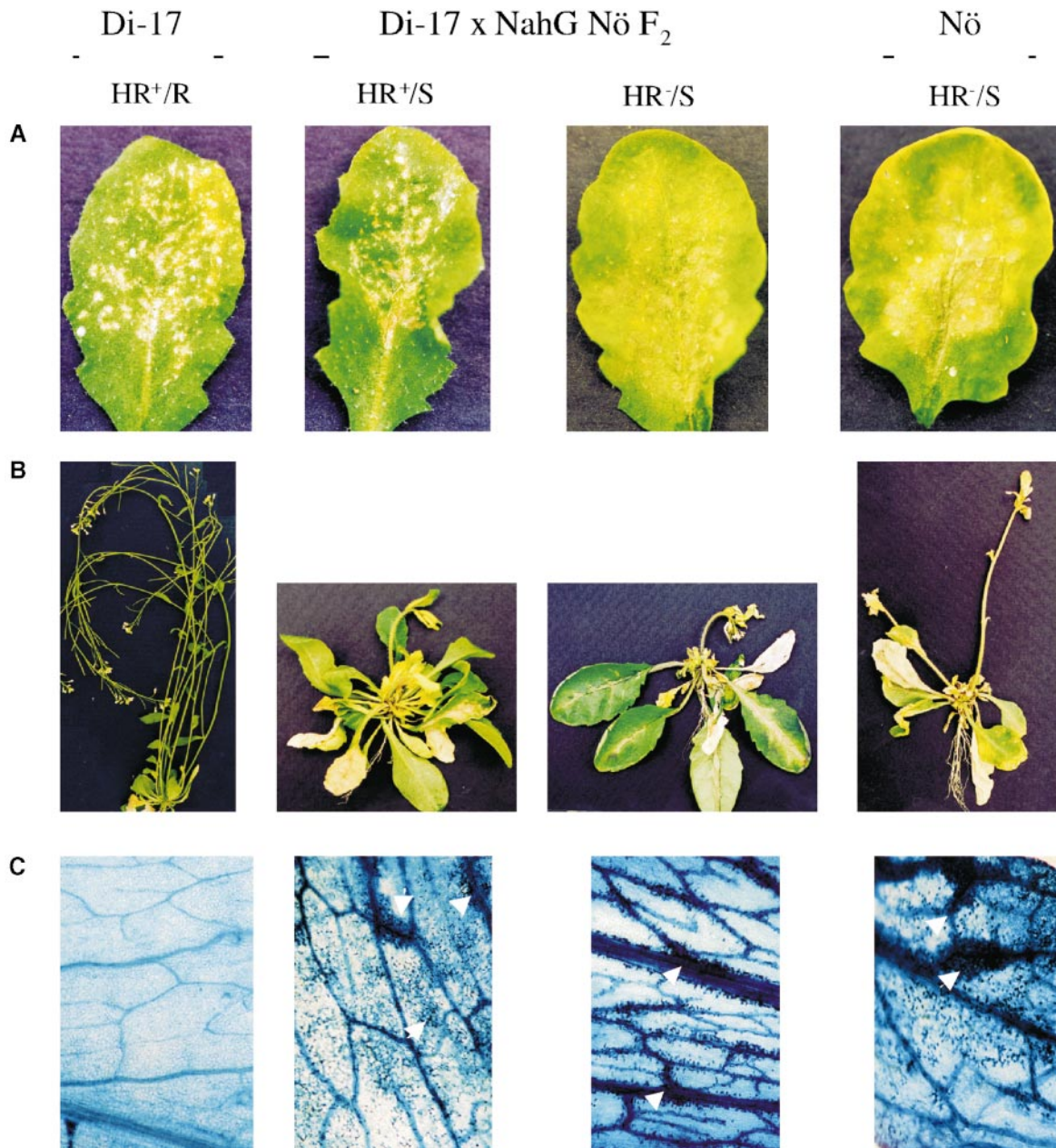


Figure 4. Morphological Phenotypes of TCV-Infected Di-17, Nö, and F₂ Plants from a Di-17 × NahG Nö Cross.

(A) TCV-inoculated leaves at 4 DPI. Both of the F₂ plants shown contain at least one allele of *HRT*. The HR⁺ F₂ plant lacks the *nahG* transgene, whereas the HR⁻ plant contains the *nahG* transgene, which leads to suppression of HR.

(B) The morphological phenotypes of the corresponding TCV-inoculated plants at 14 DPI. Except for the Di-17-resistant parent (R, resistant), the rest of the plants show severe crinkling of their leaves and stunted, drooping bolts (S, susceptible).

(C) This series shows systemic cell death induced by TCV in the corresponding TCV-infected susceptible plants. Uninoculated leaves were removed from the bolt at 14 DPI and stained with trypan blue. The leaves of the plants exhibiting disease symptoms show intensely stained areas of dead cells (marked by arrowheads), which are concentrated around the veins and gradually spread to the rest of leaf. Cell death was not evident in the uninoculated leaves of resistant Di-17 infected with TCV (left).

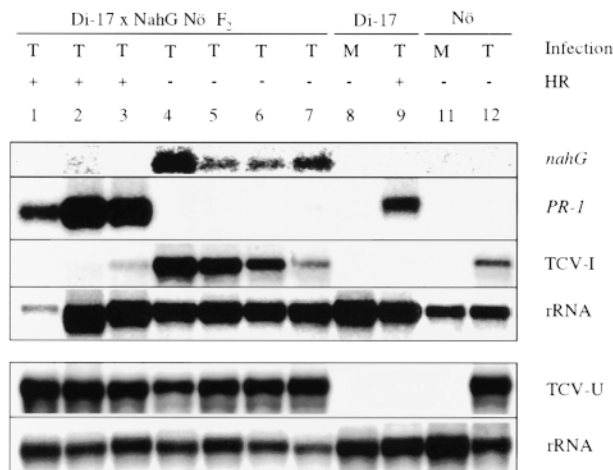


Figure 5. *PR-1* Expression and Systemic Spread of TCV in Di-17 × *nahG* F₂ Plants.

F₂ plants derived from a Di-17 × NahG Nö cross and the Di-17 and Nö parents were TCV (T)- or mock (M)-inoculated and scored for the presence (+) or absence (–) of an HR at 4 DPI. RNAs extracted at 4 DPI from the inoculated leaves of three HR⁺ and four HR[–] F₂ plants (chosen at random) and their parents were analyzed for the expression of *PR-1*, the *nahG* transgene, and the replication of TCV in the inoculated leaves (TCV-I) (1 to 12). The systemic spread and replication of TCV in the uninoculated tissue (TCV-U) were determined by analyzing RNA extracted from bolt tissues at 10 DPI. RNA extracted from the systemic tissues was run on a separate gel, and rRNA was used as an internal control for gel loading and transfer for both blots.

by way of *NPR1*-dependent and -independent pathways (Figure 7). Because ethylene and JA are the two other major signals for activation of defense responses after pathogen attack, we asked whether *PR-1* expression after TCV inoculation was affected by the *etr1-1* or *coi1-1* mutations. Analysis of at least 10 *HRT* F₂ plants insensitive to either ethylene or JA (Figure 9) failed to detect any important difference in the amounts of TCV-induced *PR-1* expression in comparison with that observed in similar F₂ progeny from a cross between Di-17 and Col-0. Thus, the pathway leading to *PR-1* expression after TCV infection appears to require neither ethylene nor JA but only SA.

DISCUSSION

In this article, we demonstrate that the dominant *HRT* allele is necessary and sufficient for development of an HR after TCV infection. *HRT* also is required for resistance to TCV; however, the results of our genetic analyses argue that a homozygous recessive allele of a second unlinked gene, termed *rrt*, is also required. *HRT* was recently cloned and shown to encode a classic leucine zipper/nucleotide binding

site/leucine rich repeat-containing protein (Cooley et al., 2000). In contrast, the structure and function of *rrt* are unknown. The recessive *rrt* allele found in Di-17 plants could encode a protein that is unable to suppress an active resistance pathway. This situation appears to exist in wheat, where an allele of the *Lr22* locus confers resistance to stem rust by encoding a nonfunctional form of protein that otherwise suppresses resistance conferred by other *R* genes (Dyck, 1987). Alternatively, the recessive *rrt* allele might encode a protein that directly or indirectly interacts with *HRT* or a downstream *HRT*-activated factor to transduce the signal leading to TCV resistance. In this scenario, the recessive nature of the *rrt* allele could be explained if it encodes a protein that functions as a multimer, and the allele carried by the susceptible ecotypes encodes a dominant-negative variant that destroys the activity of a multimeric complex. We currently are unable to distinguish between these possibilities.

Many studies have demonstrated that SA is a critical signal for the activation of defense responses and disease resistance (Durner et al., 1997; Dempsey et al., 1999). Indeed, although susceptibility to TCV was unaffected by treatment with exogenous SA or in mutants exhibiting high constitutive amounts of SA and *PR* gene expression, both HR development and TCV resistance were blocked by the presence of *nahG*-encoded salicylate hydroxylase, which prevents accumulation of SA (Table 3 and Figures 2 to 5). The possibility that the inability to accumulate SA reduces the size of TCV-induced lesions (resulting in micro-HR formation rather than loss of an HR) was ruled out by trypan blue staining of TCV-inoculated leaves from NahG^{+/–} *HRT*[–] F₂ progeny (data not shown). These results argue that SA alone is insufficient to confer resistance to TCV in the absence of *HRT*; it is required, however, for the *HRT*-mediated activation of an HR and resistance.

Because *NPR1* encodes a critical component of the SA-mediated signal transduction pathway leading to *PR* gene expression and disease resistance (Cao et al., 1994; Delaney et al., 1995; Glazebrook et al., 1996; Shah et al., 1997), we tested whether mutations in this gene would affect *PR* expression, HR development, or disease resistance after TCV infection. Plants carrying the *npr1-1* or *npr1-5* mutation exhibited delayed and decreased expression of *PR-1* after TCV infection. In contrast, *PR-1* induction was completely inhibited in TCV-infected plants expressing the *nahG* transgene. Thus, *PR-1* expression appears to be regulated by both an SA-dependent, *NPR1*-independent pathway and an SA- and *NPR1*-dependent pathway (Figure 10). Earlier studies using *npr1* mutants and bacterial pathogens also suggested the existence of an *NPR1*-independent pathway leading to *PR* expression (Glazebrook et al., 1996; Shah et al., 1997; Reuber et al., 1998).

In contrast, neither HR development nor resistance to TCV was altered in *npr1-1* or *npr1-5* mutant plants. Consistent with these results, the HR was shown to develop normally in *npr1-1* mutant plants infected with an avirulent bacterial pathogen (Cao et al., 1994). However, these same

Table 4. Segregation of the HR and Resistance to TCV in a Cross between Di-17 and *npr1-1*, *npr1-5*, *etr1-1*, or *coi1-1* Mutants

Cross ^a	Generation	Total No. of Plants	Genotype at <i>NPR1</i> , <i>ETR1</i> or <i>COI1</i> Loci ^b	Phenotype of Plants ^c				χ^2 ^d	P ^e
				HR ⁺ /S ^f	HR ⁺ /R ^g	HR ⁻ /S	HR ⁻ /R		
Di-17 × <i>npr1-1</i> (Col-0)	F ₁	2	<i>NPR1/npr1-1</i> (2)	2	0	0	0		
	F ₂	121	<i>npr1-1/npr1-1</i> (15)	11	2	2	0		
Di-17 × <i>npr1-5</i> (Nö)	F ₂	237	<i>npr1-5/npr1-5</i> (41)	22	9	10	0	0.274	>0.8
	F ₁	4	<i>ETR1/etr1-1</i> (4)	4	0	0	0		
<i>etr1-1</i> (Col-0) × Di-17	F ₂	102	<i>ETR1/etr1-1</i> plus <i>etr1-1/etr1-1</i> (74)	44	14	16	0	0.462	>0.8
	F ₁	4	<i>COI1/coi1-1</i> (4)	4	0	0	0		
<i>coi1-1</i> (Col-0) × Di-17	F ₂	137	<i>coi1-1/coi1-1</i> (38)	21	6	11	0	0.413	>0.8

^a The pollen-accepting plant is indicated first and the pollen donor second.

^b The genotype at the *NPR1*, *COI1*, and *ETR1* loci were determined by CAPS analysis. The total number of plants of the given genotype that were analyzed for the HR and resistance to TCV is given in parentheses.

^c CAPS analysis indicated that all TCV-resistant plants contained at least one copy of the dominant *HRT* allele, whereas all HR⁻-susceptible plants were homozygous for the recessive *hrt* allele. Except for the Di-17 × *npr1-1* cross, which showed skewed segregation of the *HRT* locus similar to the Di-17 × Col-0 cross (Table 2), the rest of the other crosses showed 1:2:1 Mendelian segregation of the *HRT* locus (data not shown).

^d Calculation based on a 9:3:4 ratio.

^e Two degrees of freedom.

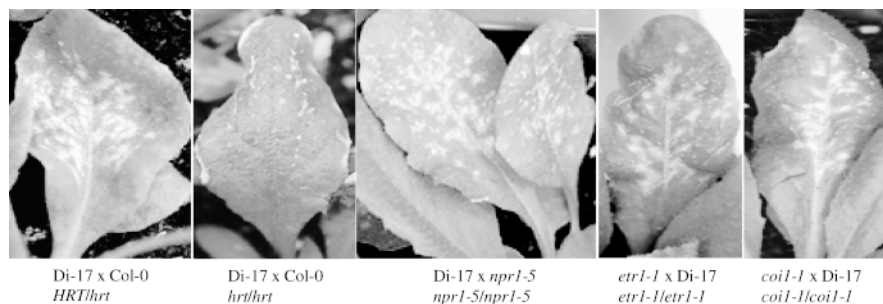
^f S, susceptible; disease symptoms include crinkling of leaves and drooping of the bolt.

^g R, resistant; no disease symptoms.

npr1 mutations caused increased susceptibility to bacterial and fungal pathogens (Cao et al., 1994; Shah et al., 1997). Given that both of the *npr1* mutant alleles used in this study contain a point mutation in the ankyrin repeats, perhaps they fail to disrupt a function necessary for HR formation and TCV resistance. Alternatively, these phenomena may be mediated by an *NPR1*-independent pathway that is distinct from the *NPR1*-dependent pathways leading to fungal and bacterial resistance (Figure 10).

Some precedence for an *NPR1*-independent viral resistance pathway comes from the studies of Carr and col-

leagues (Chivasa et al., 1997; Chivasa and Carr, 1998; Murphy et al., 1999), who demonstrated that in tobacco, resistance to TMV is mediated by an SA-dependent pathway that appears to require AOX. Strikingly, although resistance to TMV is blocked by salicylhydroxamic acid (SHAM), an inhibitor of AOX activity, the TMV-induced activation of *PR* expression is not. In addition, SHAM treatment does not affect resistance to a bacterial or a fungal pathogen (Chivasa et al., 1997). Thus, tobacco appears to contain at least two distinct SA-regulated defense pathways. One pathway presumably is *NPR1* dependent and mediates *PR*

**Figure 6.** The HR in TCV-Inoculated Leaves of F₂ Progeny from Various Crosses.

A comparison (at 4 DPI) of TCV-inoculated leaves from F₂ progeny derived from the crosses between Di-17 and wild-type Col-0, *npr1-5*, *etr1-1*, or *coi1-1* plants. The F₂ progeny from the crosses between Di-17 and *npr1-5*, *etr1-1*, or *coi1-1* were homozygous for the mutant alleles and had at least one copy of the *HRT* gene. The small specks seen on the inoculated leaves of the Di-17 × Col-0 *hrt/hrt* F₂ plant are damaged tissue or dried inoculation buffer. Those are also seen on mock-inoculated plants (data not shown; Dempsey et al., 1993).

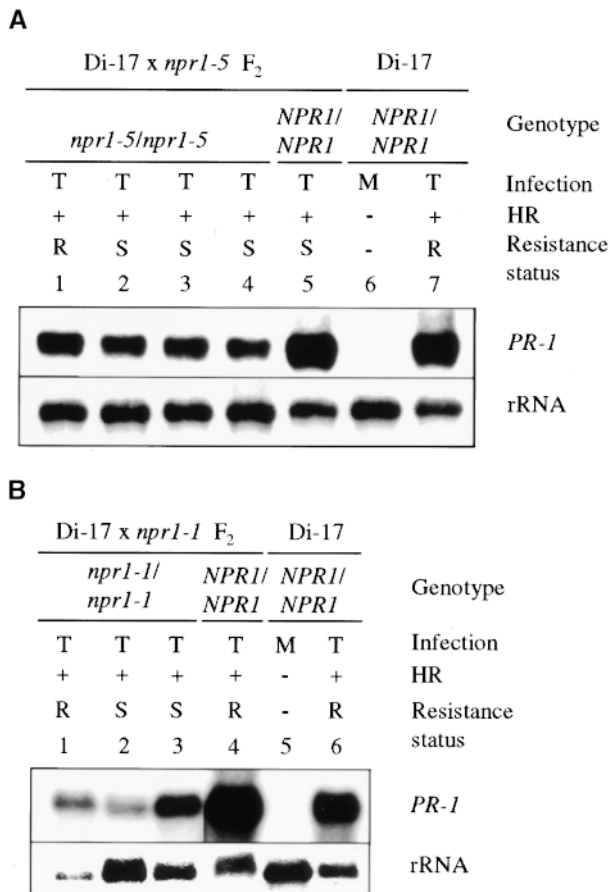


Figure 7. *PR-1* Expression in F₂ Progeny from the Crosses between Di-17 and *npr1* Plants.

(A) F₂ plants from a Di-17 × *npr1-5* cross.

(B) F₂ plants from a Di-17 × *npr1-1* cross.

The resistant Di-17 parents were inoculated with TCV (T) or inoculation buffer (M) and monitored for the presence (+) or absence (–) of an HR at 4 DPI. The plants were subsequently scored as resistant (R) or susceptible (S) at 14 DPI, based on the absence or presence of disease symptoms, respectively. The genotype at the *NPR1* locus was determined by CAPS analysis. RNA gel blot analysis was performed with RNA extracted from the inoculated leaves at 4 DPI. The blots were hybridized with probes for *PR-1* and rRNA (1 to 7 in [A] and 1 to 6 in [B]), with the latter serving as an internal control for gel loading and transfer.

expression and resistance to bacterial and fungal pathogens, whereas the other is probably *NPR1* independent and activates resistance to a virus.

In addition to SA, the secondary signals ethylene and JA have been implicated in the induction of various defense genes and the activation of resistance to certain pathogens (Xu et al., 1994; Epple et al., 1995; Penninckx et al., 1996, 1998; Dong, 1998; Pieterse and Van Loon, 1999). Thus, their

role in establishing resistance to TCV was assessed. The observation that the ethylene- or JA-inducible defense genes *PDF1.2* and *THI2.1* are not expressed in TCV-infected Di-17 plants raised the possibility that neither signal is involved in activating TCV resistance. This conclusion was confirmed by genetic studies with ethylene (*etr1-1*)- and JA (*coi1-1*)-insensitive mutants. Neither mutation affected the *HRT*-mediated development of an HR (Figure 6) or resistance to TCV (Table 4 and Figure 8). In addition, these mutations had no detectable effect on *PR-1* gene induction by TCV (Figure 9). However, because SA is the primary signal for HR development and *PR-1* expression, small contributions of ethylene or JA to these phenomena may have gone undetected in these studies.

In conclusion, we have identified a new gene, designated *RRT*, whose recessive allele works in conjunction with *HRT* to signal TCV resistance by way of an SA-dependent pathway that is independent of *NPR1*, ethylene, and JA (Figure 10). In view of these features, the TCV resistance pathway differs from the SA-dependent, *NPR1*-dependent and the SA-independent, ethylene- or JA-dependent pathways involved in activating resistance to bacterial and fungal pathogens. Strikingly, however, the *HRT/rrt*-mediated resistance pathway shares many similarities with the SA-dependent, ethylene-independent *N* gene-regulated pathway for TMV resistance in tobacco (Knoester et al., 1998; Murphy et al., 1999). These discoveries provide further evidence that viral resistance in plants is activated via a mechanism distinct from those used for other microbial pathogens. Future studies should help clarify the relationship between the two viral resistance pathways as well as provide new insights into how plants protect themselves from pathogens, particularly viruses.

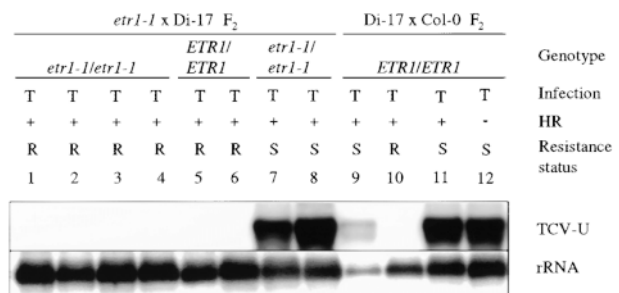


Figure 8. Systemic Spread of TCV in *etr1-1* × Di-17 F₂ Plants.

F₂ plants from an *etr1-1* × Di-17 or a Di-17 × Col-0 cross were inoculated with TCV (T) and scored for the presence (+) or absence (–) of an HR. The genotype at the *ETR1* locus was determined by CAPS analysis. The plants were scored as resistant (R) or susceptible (S) at 14 DPI, based on the absence or presence of disease symptoms, respectively. RNA was extracted from the uninoculated bolt tissues (TCV-U) at 14 DPI and analyzed for the presence of viral genome (1 to 12). This blot also was probed for rRNA as an internal control for gel loading and transfer.

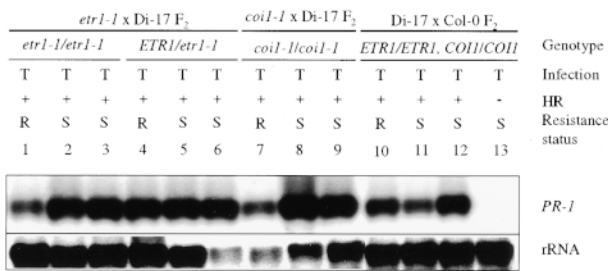


Figure 9. *PR-1* Expression in *etr1-1* × Di-17, *coi1-1* × Di-17, and Di-17 × Col-0 F₂ Plants.

F₂ plants from crosses between Di-17 and *etr1-1*, *coi1-1*, or Col-0 plants were inoculated with TCV (T) and scored for the presence (+) or absence (–) of an HR at 4 DPI. The genotype at the *ETR1* or *COI1* locus was determined by CAPS analysis. The plants were scored as resistant (R) or susceptible (S) at 14 DPI, based on absence or presence of disease symptoms, respectively. RNA was extracted from the inoculated leaves at 4 DPI and analyzed for *PR-1* expression (1 to 13). The blot was also probed for rRNA as an internal control for gel loading and transfer.

METHODS

Conditions for Plant Growth and Viral Infections

Plants (*Arabidopsis thaliana*) were grown in growth chambers as described by Shah et al. (1999). Transcripts synthesized in vitro from a cloned cDNA of turnip crinkle virus (TCV) genome using T7 RNA polymerase were used for viral infections (Dempsey et al., 1993; Oh et al., 1995). For inoculations, the viral transcript was suspended at a concentration of 0.05 μg/μL in inoculation buffer, and the infection was performed as described earlier (Dempsey et al., 1993). The hypersensitive response (HR) was determined visually 3 to 5 days post-inoculation (DPI). Tissues (leaves or bolt) were removed at various times for RNA extraction and analysis of defense gene activation and viral replication by RNA gel blot hybridization.

Histochemistry and Microscopy

Microscopic visualization of HR was conducted by staining TCV-inoculated leaves at 3 to 4 DPI with trypan blue. Samples were processed and analyzed as described by Bowling et al. (1997). Systemic cell death was studied by staining the uninoculated leaf at 14 DPI.

Chemical Treatment of Plants

Three-week-old plants were sprayed and subirrigated with a solution of 500 μM salicylic acid (SA). Control plants were treated with water, and at 2 DPI three leaves per plant were inoculated with TCV RNA.

RNA Extraction and Gel Analysis

Small-scale RNA extractions was performed with TRIzol reagent (Gibco BRL, Rockville, MD), according to the manufacturer's instructions. RNA

gel blot analysis and synthesis of random primed probes were performed as described earlier (Shah et al., 1999). RNA gel blot hybridization was performed as described previously (Kachroo et al., 1995).

Cleaved Amplified Polymorphic Sequence Analyses for *HRT*, *NPR1*, *ETR1*, and *COI1*

The genotypes of the F₂ plants at the *NPR1*, *ETR1*, *COI1*, and *HRT* loci were determined by conducting cleaved amplified polymorphic sequence (CAPS) analysis. The polymerase chain reaction (PCR)

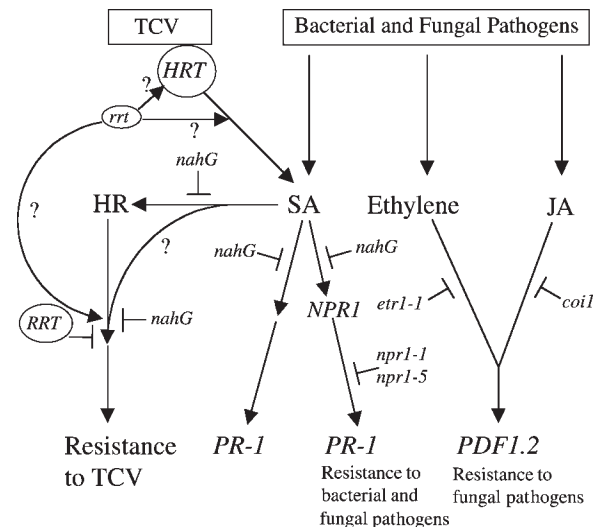


Figure 10. Model for Induction of the HR and Resistance to TCV.

Inoculation of TCV on resistant *Arabidopsis* results in an HR at the point of inoculation and the accumulation of SA (Dempsey et al., 1997). Development of the HR is mediated by the dominant *HRT* gene. Resistance is regulated by *HRT* and a second gene, *RRT*. The product of the dominant *RRT* allele or alleles present in susceptible ecotypes may act as a suppressor (T-bars) of the *HRT*-mediated resistance pathway downstream of SA and HR. Alternately, the protein encoded by the recessive *rrt* allele in Di-17 may directly or indirectly interact with *HRT* or a component downstream in the *HRT*-mediated defense pathway to facilitate transduction of the signal leading to TCV resistance. Although *HRT* is required for resistance, it is unclear if the HR is necessary also (question marks). Initiation of the HR and the signaling steps leading to resistance are blocked (T-bars) in plants expressing the *nahG* gene encoding salicylate hydroxylase. SA-mediated signaling leading to *PR-1* expression is transduced by both *NPR1*-dependent and *NPR1*-independent pathways, which are blocked by the expression of *nahG*. The *NPR1*-dependent pathway, utilized for the activation of resistance to some bacterial and fungal pathogens, is not required for the HR or resistance to TCV. Ethylene- or JA-mediated signaling that leads to *PDF1.2* expression and resistance to some fungal and bacterial pathogens is not necessary for the HR or resistance to TCV because mutations resulting in insensitivity to ethylene (*etr1-1*) or JA (*coi1-1*) do not affect either of these phenotypes. Thus, the pathways required for resistance to TCV differ from those for bacterial or fungal pathogens.

primers used for amplifying the *NPR1* locus were as described by Shah et al. (1999), and the plants containing *npr1-1* and *npr1-5* alleles were identified by digesting the amplified product with *Nla*III or *Nla*IV, respectively. Because the *etr1-1* (Chang et al., 1993) mutation does not lead to an alteration of any restriction site, we used the dCAPS technique (Neff et al., 1998) to generate polymorphism between the wild type and *etr1-1* alleles. PCR primers were designed to amplify a 100-bp region encompassing the mutant base, where the reverse primer contained two altered bases at the 3' end such that the presence of the mutant base in *etr1-1* allele results in loss of an *Apa*LI restriction site. Therefore, digestion of the PCR product from plants homozygous for the wild-type allele yielded two bands at 75 and 25 bp, whereas the digested PCR product amplified from *etr1-1* DNA generated only a single band at 100 bp. The PCR reaction (*ETR1* Fwd, 5'-CTTTGTGAAGAAATCAGCCGTGT-3'; *ETR1* Rev, 5'-CCATAAGTTAATAAGATGAGTTGGTGCA-3') was performed at an annealing temperature of 56°C for 50 cycles. The PCR-based analysis to determine the genotype at the *COI1* locus was done as described by Xie et al. (1998). The genotype at the *HRT* locus was determined by producing a 950-bp PCR product, followed by digestion with *Alu*I (Cooley et al., 2000). After digestion, the PCR product of Dijon (Di-17) *HRT* allele generated two bands of ~100 and 850 bp. The 850-bp fragment was further cleaved into two fragments of 300 and 550 bp when the PCR products were derived from *hrt/hrt* Columbia (Col-0) or Nössen (Nö) ecotypes.

Genetic Analysis

Reciprocal crosses of Di-17 plants with Nö plants homozygous for the *nahG* transgene were performed. The success of the crosses was determined by CAPS analysis and by analyzing expression of the *nahG* gene. To facilitate easy identification of F₂ plants containing the *nahG* transgene, two PCR primers (5'-GGCTTGCATCGGT-ATCGTCGGC-3' and 5'-GCCATGGGCCCGATAGGCTTCTCG-3') were designed to yield a 500-bp amplification product. PCR amplification of *nahG* was performed at an annealing temperature of 60°C for 35 cycles. Crosses of Di-17 with *npr1-1* or *npr1-5* plants were performed by using pollen from the mutant plants to pollinate the Di-17 flowers. The crosses with *etr1-1* and *coi1-1* plants were made by pollinating the flowers from these plants with pollen from Di-17. The F₁ plants were analyzed with *NPR1*-, *ETR1*-, or *COI1*-specific CAPS and with random CAPS or simple sequence length polymorphism (SSLP) markers from the genome to ensure that progeny indeed resulted from the crosses. The F₂ plants were first analyzed by gene-specific CAPS markers and subsequently were inoculated with TCV. DNA for PCR was isolated either from leaf or bolt tissue by the method of Konieczny and Ausubel (1993). CAPS or SSLP amplifications were performed as described earlier (Konieczny and Ausubel, 1993; Bell and Ecker, 1994).

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NOTE ADDED IN PROOF

Interestingly, *RPP8*-dependent resistance to *Peronospora parasitica* has recently been shown to be largely independent of SA as well as *NPR1* (McDowell, J.M., Dangl, J.L., and Holub, E.B. [2000]. *Plant J.*, in press).