Resistance and Susceptible Responses of *Arabidopsis thaliana* to Turnip Crinkle Virus

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ABSTRACT

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We describe the first system in which a hypersensitive response (HR) is observed after viral infection of *Arabidopsis thaliana*. Most ecotypes of *Arabidopsis* are highly susceptible to turnip crinkle virus (TCV); however, we developed two lines from the ecotype Dijon that exhibit dramatic differences in TCV susceptibility. Inoculation of the resistant line, Di-17, resulted in necrotic lesion formation on the inoculated leaves. Virus was restricted to these lesions, and no disease symptoms were observed on the uninoculated portions of most plants. Genes encoding the acidic pathogenesis-related (PR) proteins PR-1, PR-2, and PR-5 and the basic form of PR-3 were induced rapidly in the inoculated leaves, whereas upper, uninoculated leaves accumulated mRNA for these genes at slightly later times and to lower levels. In contrast, the susceptible line, Di-3, did not express a HR. TCV was detected throughout these plants by 3 days post-inoculation. Systemic disease symptoms appeared a few days later and became increasingly severe until plant death. PR gene induction in both inoculated and uninoculated leaves occurred later and to a lesser extent than in Di-17. Several developmental and environmental conditions also were assessed for their effect on the TCV-resistance levels exhibited by Di-17 and Di-3.

Plant–pathogen interactions have been studied for many years and yet the mechanism(s) by which plants perceive pathogen attack and initiate a resistance response is still poorly understood. One of the better characterized plant–pathogen interactions utilizes tobacco mosaic virus (TMV) and resistant and susceptible cultivars of tobacco. After TMV inoculation, resistant tobacco cultivars, such as Xanthi-nc (NN) or Samsun NN, express a hypersensitive response (HR) that results in the formation of necrotic lesions and the prevention of viral movement to the uninoculated portions of the plant (23).

Several families of pathogenesis-related (PR) genes also are induced during the resistance response (4,9,21). Although the functions of some of these proteins are still unclear, several have been shown to have antimicrobial activities, i.e., chitinases (18) and glucanases (16), that can hydrolyze fungal and bacterial cell walls. Initially, these PR genes are expressed in the inoculated leaves; however, at later times they also are induced in the uninoculated portions of the plant (3,7,40). This systemic expression correlates with a phenomenon known as systemic acquired resistance (SAR) in which the entire plant exhibits enhanced levels of resistance to a secondary challenge by a wide variety of unrelated pathogens.

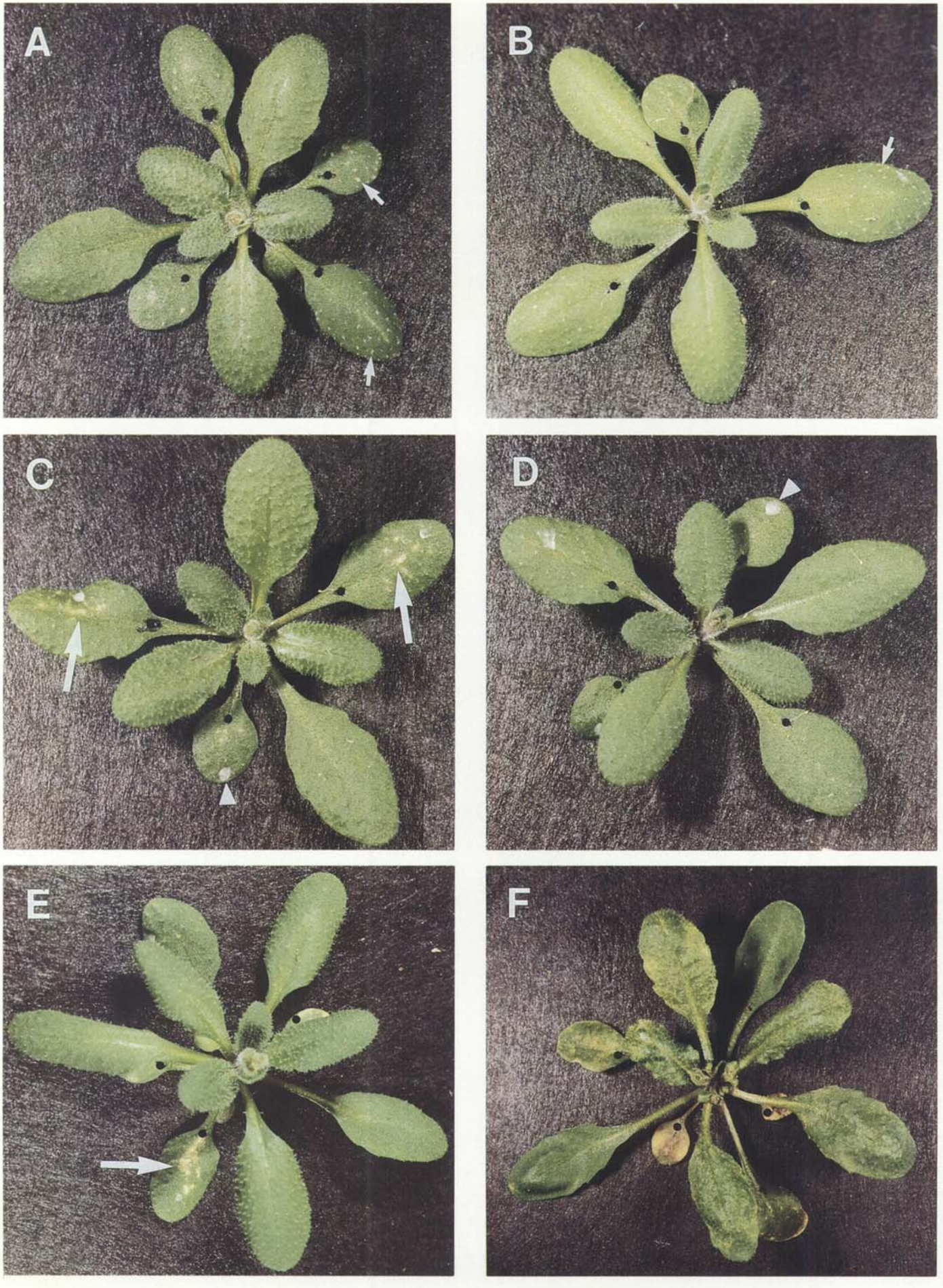
The pathway by which HR and, subsequently, SAR are activated in resistant tobacco cultivars after TMV inoculation has yet to be elucidated. There are strong indications that salicylic acid is a signaling molecule involved in the induction of SAR (22,30). However, in spite of extensive efforts, neither the N locus, which confers resistance, nor other genes in the signal transduction pathway have been isolated. This is due in part to the complicated genetics and large genome size of tobacco.

To simplify the search for components of the resistance pathway, several labs are using *Arabidopsis* as a model host plant. The small genome and rapid life cycle of *Arabidopsis* simplifies genetic manipulation at both the plant breeding and molecular levels (26,27). At present, a detailed RFLP (restriction fragment length polymorphism) map exists (5,29), and many genes, including several for PR proteins, have been cloned and sequenced (12,31,39).

A number of bacterial (10–12,36,38,41), fungal (17), viral (14,19, 20,24,35,37), and nematode (34) pathogens of *Arabidopsis* have been identified. Extensive efforts are currently underway to identify the plant genes involved in the resistance response to pathovars of *Pseudomonas* and *Xanthomonas*, and loci that confer resistance to these pathogens have been identified (11,38). The interactions between *Arabidopsis* and viral or fungal pathogens have been considerably less well studied. We report here the characterization of the interaction between *Arabidopsis thaliana* (L.) Heynh. and turnip crinkle virus (TCV).

TCV is a small icosahedral virus that infects a variety of cruciferous plants including *Arabidopsis*. It contains a single positive-strand RNA genome of 4 kb (28) that encodes a coat protein, two movement proteins, and two proteins required for viral replication (13). In addition, TCV-M, the isolate used for these studies, is associated with a variety of satellites, only one of which, satellite C, has been demonstrated to increase the severity of viral symptoms in turnip and *Arabidopsis* (1,20). The other two satellites associated with TCV-M, satellites D and F, are asymptomatic. Although many *Arabidopsis* ecotypes are susceptible to TCV-M (20), Simon et al (35) have identified the ecotype Dijon, Di-0, as partially resistant. From the Di-0 seedline, we have further isolated TCV-susceptible (Di-3) and -resistant (Di-17) lines of Dijon.

In this paper, we describe the characterization of the responses of these two newly isolated lines of Dijon to TCV. Unlike the previously reported Di-0 ecotype, the Di-17 plants consistently express a HR in response to TCV inoculation; necrotic lesions develop on the inoculated leaves, and the virus is restricted to the cells within or immediately surrounding the lesions. These plants also express several families of PR genes in both the inoculated and uninoculated leaves. The Di-3 line, in contrast, is completely susceptible to TCV. No HR is exhibited by these plants, induction of the PR genes occurs later and to a lesser extent, and the plants rapidly become systemically infected. The dramatically different responses of these two closely related lines of Dijon to TCV will provide new opportunities to isolate the components of the resistance pathway.


A
B
C
D
E
F

MATERIALS AND METHODS

Growth conditions and inoculation procedures. *Arabidopsis* was grown on Pro Mix BX (Premier Brands Inc., New Rochelle, NY) under 14,500 lx during 14-h days at 21 C, except where indicated. High-intensity light conditions were achieved by growing the plants under continuous light at 16,000 lx. Plants were watered by placing the pots in tubs of H_2O and were fertilized once a week with Peter's general purpose 20-20-20 N-P-K fertilizer (W. R. Grace & Co., Cambridge, MA) at 0.7 gm/L.

Plants were inoculated at the five to six partially expanded and two to three emerging leaf stage (~18 days after planting). The source of TCV was total RNA isolated from turnip plants infected with TCV-M. TCV-M contains, in addition to the viral genomic RNA, several associated satellite (Sat) RNAs, including Sat C, which increases virulence, and Sats D and F, which do not affect disease symptoms (20). This RNA was diluted to a final concentration of 0.1 mg/ml in RNA inoculation buffer consisting of 0.05 M glycine, 0.03 M K_2HPO_4, 0.02% bentonite, and 1% celite. A glass rod was dipped in the inoculum and gently rubbed over the surface of the first four leaves. Mock inoculations were performed in an analogous manner, using inoculation buffer without TCV. All inoculated leaves were marked with a nontoxic marker. Plants were returned to their initial growth conditions, except where indicated. On designated days, leaves were detached and frozen in liquid N_2, or plants were scored for disease symptoms. All experiments described in this paper were performed independently two to four times.

RNA Extraction. RNA extractions were performed essentially as described by Berry et al (2). The only modification was the addition of a phenol extraction after the first ethanol precipitation, followed by another ethanol precipitation before precipitating the RNA with LiCl. This extra phenol extraction removed nucleases and other contaminants, significantly improving the quality of the RNA.

Northern analyses. All Northern analyses shown here were done with RNA harvested from one set of plants, although the experiments were repeated several times. RNA was fractionated on 1% agarose formaldehyde gels according to Sambrook et al (33) with 5 μg of RNA per lane. Ethidium bromide was added to the RNA samples before loading. Transfer of RNA to Nytran membrane was performed according to the manufacturer's protocols (Schleicher and Schuell, Inc., Keene, NH). Probes were prepared as follows. The TCV genomic cDNA probe was isolated from pT7TCV-7 by *Sma*I digestion (35) followed by gel fractionation. The basic chitinase (PR-3) probe was prepared from pMON8817 as a 1.5-kb *Bgl*II/*Eco*RI fragment (31). The PR-2 probe was a 246-bp *Hin*fI fragment from pBG2 (12) that specifically hybridizes to the acidic β-glucanase-2 (BG-2) gene but not the BG-1 or BG-3 genes. (Preliminary experiments demonstrated that neither the BG-1 nor the BG-3 genes were significantly induced in response to TCV inoculation.) All other clones were simply linearized before labeling. The PR-1 and PR-5 clones were linearized with *Eco*RI (39); pMON10829, the acidic chitinase clone (PR-3), was linearized with *Bgl*II before labeling (31); PSKPAL, the clone containing PAL (phenylalanine ammonia lyase), was linearized with *Eco*RI (12); and a soybean ribosomal RNA clone, pKDR1, was linearized with *Eco*RI (obtained from J. L. Key, University of Georgia, Athens). Probes were labeled using Prime-It (Stratagene, La Jolla, CA). Blots were prehybridized and then hybridized according to Church and Gilbert (6) with 10^6–10^7

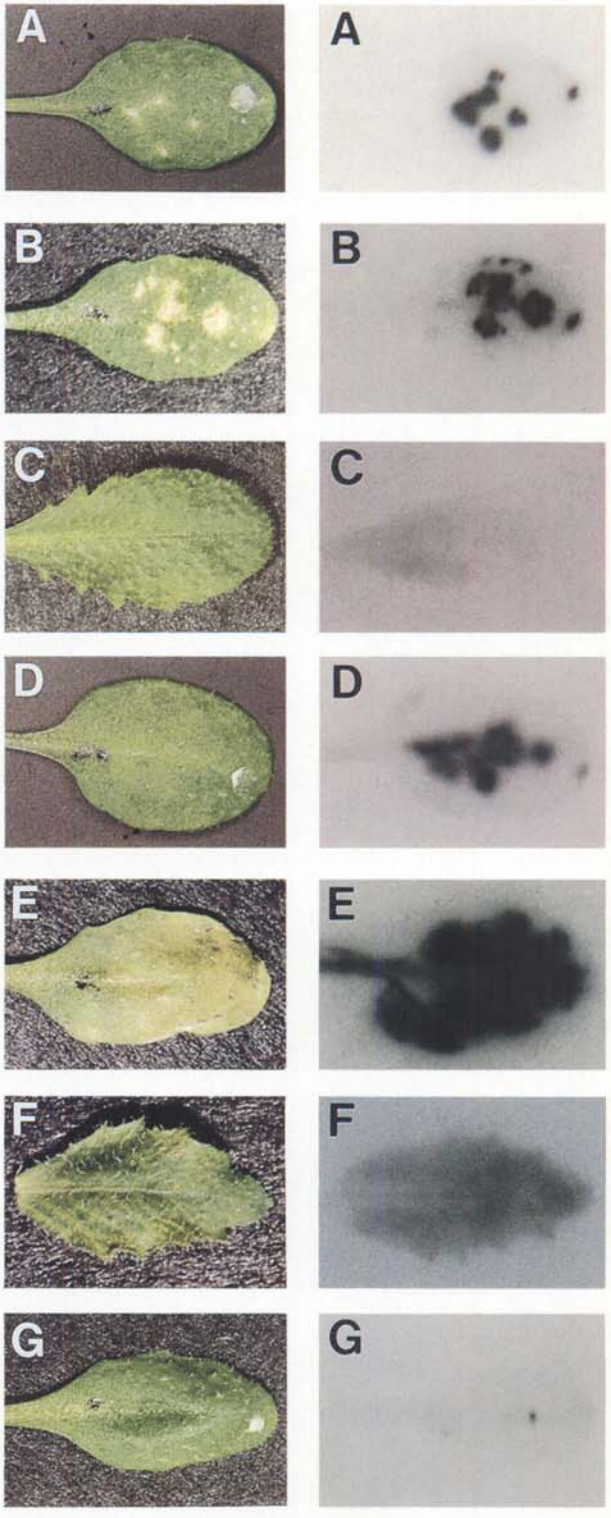


Fig. 2. In situ localization of turnip crinkle virus (TCV) in inoculated and uninoculated leaves of Di-17 and Di-3 plants. At the designated times post-inoculation, inoculated and uninoculated leaves were photographed, hybridized with the TCV probe, and autoradiographed. Pictures of the leaves (white letters) and their corresponding autoradiographs (black letters) are shown. **A,** Three days post-inoculation (dpi) inoculated leaf of a TCV-inoculated Di-17 plant; **B,** 6 dpi inoculated leaf of a TCV-inoculated Di-17 plant; **C,** 6 dpi uninoculated leaf of a TCV-inoculated Di-17 plant; **D,** 3 dpi inoculated leaf of a TCV-inoculated Di-3 plant; **E,** 6 dpi inoculated leaf of a TCV-inoculated Di-3 plant; **F,** 6 dpi uninoculated leaf of a TCV-inoculated Di-3 plant; **G,** 6 dpi inoculated leaf of a mock-inoculated Di-17 plant.

◀ **Fig. 1.** Symptoms exhibited by Di-17 and Di-3 in response to turnip crinkle virus (TCV) inoculation. **A,** Three days post-inoculation (dpi) mock-inoculated Di-17; **B,** 3 dpi mock-inoculated Di-3; **C,** 3 dpi TCV-inoculated Di-17; **D,** 3 dpi TCV-inoculated Di-3; **E,** 6 dpi TCV-inoculated Di-17; **F,** 10 dpi TCV-inoculated Di-3. Bolt has been removed to facilitate photography. Inoculated leaves are designated with a black dot near the base of the leaf. Damaged leaf hairs (small white arrows) and buffer spots (white arrow heads) are denoted on the inoculated leaves of both Di-17 and Di-3. Virus-induced lesions (large white arrows) are visible in **C** and **E**.

cpm/ml at 60 C. Blots were washed twice in 2× SSC (1× SSC is 0.15 M sodium chloride, 0.015 M sodium citrate, pH 7.0) at room temperature for 5 min each, twice in 2× SSC and 0.5% SDS (sodium dodecyl sulfate) at 65 C for 30 min each, and, finally, twice in 0.1× SSC at room temperature for 30 min each. Autoradiography was performed with Kodak X-OMAT AR film.

TCV localization. TCV localizations were performed with a modification of the method of Melcher et al (25). Briefly, after the leaves were photographed, they were placed in ethanol for 2–4 h at room temperature to remove chlorophyll. After clearing, the leaves were incubated in 0.1 mM NaN_3, 0.1% (w/v) SDS, and proteinase K at 0.1 mg/ml overnight at 37 C. After treatment in 0.15 N HCl for 2 min, the leaves were rinsed in 2× SSC and air-dried. The leaves were baked at 80 C for 2 h under vacuum. For prehybridization, leaves were placed in 2× SSC, 1× Denhardt's solution, and 0.1% BSA (bovine serum albumin) for 15 min at 68 C. Sheared calf thymus DNA at 1 mg/ml and TCV genome probe at 1×10^6 cpm/ml were boiled for 5 min and added to the prehybridization solution. Hybridization was carried out at 68 C overnight, followed by two washes in 2× SSC for 10 min each at 68 C. Leaves were placed between Saran Wrap layers and autoradiographed using Amersham hyperfilm MP (Amersham Corp., Arlington Heights, IL) at −70 C.

RESULTS

Establishment of TCV-resistant and -susceptible lines of the *Arabidopsis* ecotype Dijon. Preliminary experiments by Simon et al suggested that the Dijon ecotype of *Arabidopsis* was resistant to TCV (35). However, when Di-0 plants were inoculated with TCV, we observed a variety of symptoms, ranging from resistant to completely susceptible. This suggested that the original Di-0 population was heterogeneous. To obtain plants that were more homogeneous, 64 plants were allowed to self-fertilize, and their progeny were tested for TCV resistance. One of the 64 plants produced progeny that were 100% susceptible, dying by 20 days post-inoculation (dpi). This line produced only susceptible plants over two additional rounds of self-fertilization and was designated Di-3.

The remaining 63 plants produced progeny that exhibited variable levels of resistance to TCV. Seeds were isolated only from the plant producing the largest percentage of resistant progeny, and the next three generations were grown under selective pressure, i.e., infected with TCV. In each generation, only progeny that showed no systemic symptoms were allowed to self-fertilize, and their progeny were reinoculated with TCV. After five generations, the level of resistance had not changed substantially, with approximately 70% of the plants exhibiting complete resistance, i.e., development of necrotic lesions at 3 dpi with no further symptoms. However, line Di-17 has been bred free of the Di-3 type plant, as evidenced by the different responses of the two lines to viral inoculation. These newly isolated lines are morphologically very similar and appear to be closely related to the parental Di-0 plants.

Symptomology. *Arabidopsis* was infected with TCV by inoculating the oldest four leaves of plants bearing five to six partially expanded and three newly emerging leaves (~18 days post planting [dpp]). By 3 dpi, very small, lesionlike spots presumably caused by damaged leaf hairs were observed on the inoculated leaves of both mock- and TCV-inoculated plants (indicated by small arrows in Fig. 1A and B). They were clearly distinguishable from the TCV-induced lesions exhibited by Di-17 at 3 dpi. The TCV-induced lesions appeared on the inoculated leaves as silver-brown regions ~0.5 mm in diameter (indicated by large arrows in Fig. 1C). No comparable lesions appeared on the inoculated leaves of mock-inoculated plants, demonstrating that lesion formation was specific to the presence of virus (Fig. 1A and B). Over the next few days the lesions continued to expand until they reached a final size of 2–3 mm in diameter (Fig. 1E). The lesion-bearing leaves persisted on the plant, becoming chlorotic and senescing only slightly more rapidly than comparable leaves on mock-inoculated plants. The majority of the Di-17 plants showed no other symptoms; their development did not appear to be affected by TCV inoculation as they bolted, flowered, and set seed concurrently with their mock-inoculated counterparts.

Although all Di-17 plants developed TCV-induced lesions, not all remained free of further symptoms. By 8 dpi, ~5% of the Di-17 plants developed severe symptoms analogous to those exhibited by the susceptible Di-3 plants. These symptoms included crinkled new leaves and bolts that either failed to elongate or grew only a few centimeters before wilting and hooking over. When samples were collected for molecular analysis, these susceptible Di-17 plants were excluded. During the second week after TCV inoculation, another ~25% of the Di-17 plants developed mild symptoms, consisting of drooping bolt tips and, occasionally, curled siliques.

In contrast to Di-17, the susceptible Di-3 plants were asymptomatic at 3 dpi (Fig. 1D). Symptoms were first visible on these plants between 4 and 6 dpi. The inoculated leaves exhibited a mottled appearance with regions of chlorosis (Figs. 1F and 2E) that became more pronounced with time, and these leaves prematurely senesced. Systemic symptoms were first observed on the uninoculated rosette and cauline leaves of Di-3 plants at 6 dpi. These leaves developed a striped pattern with alternating bands of yellow and dark green stretching transversally across the leaf (Figs. 1F and 2F). As the infection progressed, this striping pattern became more pronounced. The young leaves also were severely crinkled and twisted and failed to elongate properly. Unlike the bolts of mock-inoculated plants, those of TCV-inoculated Di-3 plants were severely stunted. The tips of these bolts curled and produced aberrant flower heads that usually failed to set siliques. The plants from the Di-3 line were dead by 18–21 dpi.

The two lines of Dijon were inoculated at various developmental ages to assess the effect of this parameter on TCV susceptibility. This analysis indicated that increased survivability directly correlated with increasing age of both Di-17 and Di-3 plants at the time of infection. Although Di-3 plants always died in response to TCV infection, inoculation of younger plants resulted in more severe symptoms, and plants died more rapidly than did plants inoculated even 2 days later. For Di-17, the percentage of plants that developed systemic disease symptoms decreased significantly as the age of the plants at the time of inoculation increased. Inoculation at 16 dpp resulted in only 20–56% of the plants remaining resistant, whereas inoculation at 18 dpp increased the proportion of resistant plants to 65–80% and inoculation at 20 dpp resulted in resistance in 90–100% of Di-17 plants. For all of the experiments described in this paper, we chose to inoculate plants at the five- to six-leaf stage (~18 dpp). Although plants inoculated at 20 dpp exhibited higher levels of resistance, they started to bolt soon after inoculation (~24 dpp), and presumably due to their advanced stage of development, the older inoculated leaves contained very low quantities of extractable RNA, making analysis of viral replication and PR gene expression very difficult.

We have very recently discovered that a 2-wk vernalization produces synchronous germination, in contrast to nonvernalized seed, which germinates over a 3- to 5-day period. When vernalized

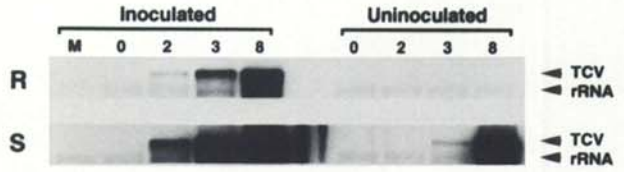


Fig. 3. Detection of viral genome in turnip crinkle virus (TCV)-inoculated Di-17 and Di-3 plants. RNA was harvested from inoculated and uninoculated leaves of TCV-inoculated Di-17 (R) and Di-3 (S) plants at 0, 2, 3, and 8 days post-inoculation (dpi). RNA also was isolated from the inoculated leaves of mock-inoculated plants (M) at 8 dpi. Five micrograms of RNA was loaded per lane, and the resulting blots were probed with a cDNA clone of the TCV genome. The TCV probe shows a low level of cross-hybridization with the abundant ribosomal RNA.

Di-17 plants are inoculated at 18 dpp, they are virtually 100% resistant. Thus, we feel that the Di-17 plants that die in response to TCV inoculation are simply younger than their resistant counterparts.

Viral replication. The extent of viral replication in both TCV-inoculated and uninoculated leaves of resistant Di-17 and susceptible Di-3 *Arabidopsis* was determined by Northern analysis. Viral RNA accumulated to detectable levels by 2 dpi in Di-17 inoculated leaves (Fig. 3) and continued to accumulate in these leaves throughout the observation period. TCV RNA was not detected in the uninoculated portion of Di-17 plants or in mock-inoculated plants.

In the susceptible Di-3 line, a different pattern of TCV accumulation was observed. The virus accumulated more rapidly and to a greater extent in the inoculated leaves and also spread systemically throughout the uninoculated regions of the plant. By 2 dpi, the TCV-inoculated leaves of Di-3 had accumulated at least twofold more TCV genomic RNA than comparable leaves of Di-17 (Fig. 3). Over the duration of the observation period, TCV genome levels in the inoculated leaves continued to increase. Systemic viral accumulation was observed first in Di-3 at 3 dpi, and the level increased rapidly throughout the observation period. Almost equivalent levels of viral RNA were detected in the inoculated and uninoculated leaves by 8 dpi.

Viral localization. In situ localizations were performed on inoculated and uninoculated leaves to determine the extent of viral movement during infection of Di-17 and Di-3 plants. After hybridizing the inoculated Di-17 leaves with the TCV probe at 3 dpi, viral genome was detected only within the lesions (Fig. 2A). Short exposures showed sharp rings of dark hybridization around the edges of the lesions, indicating that the majority of the virus was localized to the lesion perimeter. By 6 dpi, the lesions had increased in size; however, the virus was still restricted to the lesion sites (Fig. 2B). No viral RNA was detected in the uninoculated leaves of resistant plants (Fig. 2C) or in either the inoculated or uninoculated leaves of mock-inoculated control plants (Fig. 2G).

In contrast, TCV was far more mobile in the susceptible Di-3 line. Although visible lesions did not form on the inoculated leaves of Di-3 plants, by 3 dpi in situ hybridizations localized TCV RNA to discreet regions on the leaf that probably represented sites of viral entry (Fig. 2D). Unlike the hybridization pattern observed around the lesions on Di-17, the viral RNA was evenly distributed throughout these regions, and their borders appeared diffuse. No TCV could be detected in uninoculated leaves at this time. At 6 dpi, the inoculated leaves of Di-3 exhibited chlorotic spots, and the upper, uninoculated leaves had developed yellow and green stripes. In situ hybridizations of the inoculated leaves showed larger regions of strong signal, corresponding to those observed at 3 dpi, surrounded by a diffuse hybridization pattern that covered most of the leaf (Fig. 2E). Considerable quantities of viral RNA also were localized to the midveins and petioles. A strong, diffuse pattern of hybridization also was observed in the upper, uninoculated leaves, indicating that substantial viral movement and replication had occurred (Fig. 2F).

Induction of PR protein genes. The induction by TCV of the *Arabidopsis* genes for the acidic forms of PR-1, PR-2, PR-3, PR-5, and the basic form of PR-3 was monitored by Northern analysis. Phenylalanine ammonia lyase (PAL) also was assayed for induction. At the time of infection, the level of these PR mRNAs in both inoculated and uninoculated leaves of mock- and TCV-inoculated plants ranged from low to nondetectable. Many of the PR genes tested showed rapid induction in the inoculated leaves of the resistant Di-17 plants after TCV treatment. PAL and the acidic form of PR-3, however, were not induced by TCV challenge of either Di-17 or Di-3 at the times assayed (data not shown). Although induction of PAL by other pathogens has been observed (10,12), the mRNA for acidic PR-3 has not been detected in *Arabidopsis* responding to pathogen attack (31,32).

In Di-17 plants, mRNAs for acidic PR-1, PR-2, PR-5, and basic PR-3 were first detected at 2–3 dpi in the inoculated leaves. At the peak of expression, their induction over background ranged from seven- to greater than 20-fold (Figs. 4 and 5). The same PR genes also were induced in the upper, uninoculated leaves at slightly later times and to lower levels than in the inoculated leaves. Although small increases in PR mRNA were observed in mock-inoculated leaves during the observation period, this level of expression was considerably lower than that found in the corresponding TCV-inoculated leaves and was presumably caused by the stress of mechanical inoculation. The uninoculated leaves of mock-inoculated plants expressed little to no PR mRNAs (Fig. 4).

The PR mRNAs in the Di-3 plants inoculated with TCV either failed to accumulate over background or were induced at later times and to lower levels than in comparable leaves on Di-17 plants. At their maximum levels, the mRNAs in the inoculated leaves of Di-3 were two- to greater than 20-fold less than in the inoculated leaves of Di-17 (Figs. 4 and 5). Only PR-1 and PR-2 were expressed in the uninoculated leaves of Di-3, and their induction occurred at least 1 day later than the induction observed in uninoculated leaves of Di-17. Unexpectedly, the level of PR-2 mRNA increased considerably, and by 8 dpi, these leaves contained twofold more message than did the uninoculated leaves of Di-17.

High-intensity, continuous light and resistance. We found that plants grown under high-intensity (16,000 lx), continuous light exhibited increased resistance to TCV. Changing either parameter (light level or day length) individually had no effect on resistance levels; however, 16,000 lx/24 h of light (continuous light conditions) both increased resistance levels and altered plant morphology.

The morphology of plants grown under the continuous light conditions was particularly abnormal during the first 14 dpp. By the four-leaf stage, small brown, lesion-like spots had appeared on the leaves and cotyledons of both Di-17 and Di-3 (data not

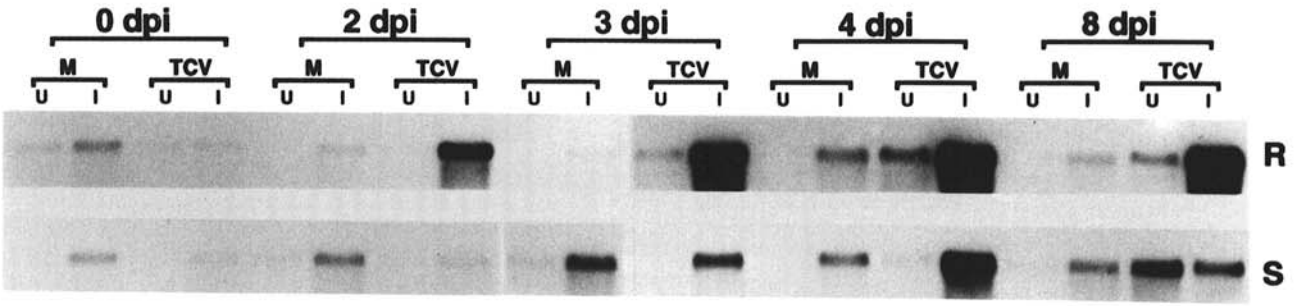


Fig. 4. Analysis of pathogenisis-related (PR) gene induction by turnip crinkle virus (TCV) infection. Northern analysis of PR-2 induction in mock- and TCV-inoculated Di-17 and Di-3 plants. At the indicated times post-inoculation, RNA was harvested from uninoculated (U) and inoculated (I) leaves of mock- (M) and TCV-inoculated Di-17 (R) and Di-3 (S) plants. Five micrograms of RNA was loaded per lane, and the resulting blots were sequentially hybridized with clones of the PR genes and a soybean ribosomal RNA clone, pKDR1. For clarity, only the autoradiogram of the PR-2 hybridized blot is presented here.

shown). As these plants developed, their leaves became a much darker green, were more curled under, and were more brittle than were those of their counterparts grown under the 14,500 lx/14-h day (normal light) conditions. In addition, the leaves of Di-17 frequently developed necrotic stripes. Continuous light hastened the development of both Di-17 and Di-3; they began to bolt around 16 dpp, much sooner than those grown under normal light conditions. After bolting commenced, these plants appeared more normal but grew more vigorously than those grown under normal light.

In addition to changes in morphology, the continuous light conditions produced increased levels of resistance in both Di-3 and Di-17. In an effort to standardize developmental age at the time of inoculation, these plants were inoculated with TCV at 14 dpp, 2–3 days prior to the onset of bolting. Although Di-3 plants still developed systemic viral infections, their symptoms were less severe, and they survived slightly longer after inoculation than did their counterparts grown under normal light conditions. In contrast, continuous light conditions increased the resistance level of Di-17 such that nearly all the plants survived viral inoculation. It was difficult to detect or distinguish viral necrotic lesions on these plants, however, due to the large number of necrotic spots already present on uninoculated plants.

The mechanism by which the continuous light condition increased resistance levels in Di-17 plants was further investigated by growing the plants initially under either continuous or normal light regimes and then switching the plants to the opposite condition at 0, 2, or 4 days before inoculation. The percentage of plants that exhibited no systemic symptoms during the ensuing 14 days after inoculation was monitored. Surprisingly, when Di-17 plants grown under normal light were switched into continuous light at the time of inoculation, the percentage of resistant plants was significantly reduced over that of plants grown only under normal light conditions (Fig. 6A). However, increasing the length of the pretreatment in continuous light prior to TCV inoculation did increase the percentage of resistant plants. A 2-day pretreatment in continuous light had little effect on the proportion of resistant plants (65%), whereas a 4-day pretreatment increased the proportion of resistant plants to 85%. The highest levels of resistance were achieved by growing the plants under continuous light conditions up to the time of inoculation.

In contrast, when plants grown under continuous light were switched to normal light conditions at the time of inoculation, no effect on resistance levels was observed compared to plants still growing under continuous light (Fig. 6B). A 2- or 4-day pretreatment in normal light caused a small reduction in resistance. Regardless of the duration of the pretreatment, all of the plants initially grown under continuous light and then under normal light conditions were considerably more resistant than plants grown only under normal light.

In an attempt to determine the basis of this increased resistance, we investigated the levels of PR messages in plants grown under continuous light and their counterparts grown under normal light (Fig. 7). Samples from untreated, mock- or TCV-inoculated Di-

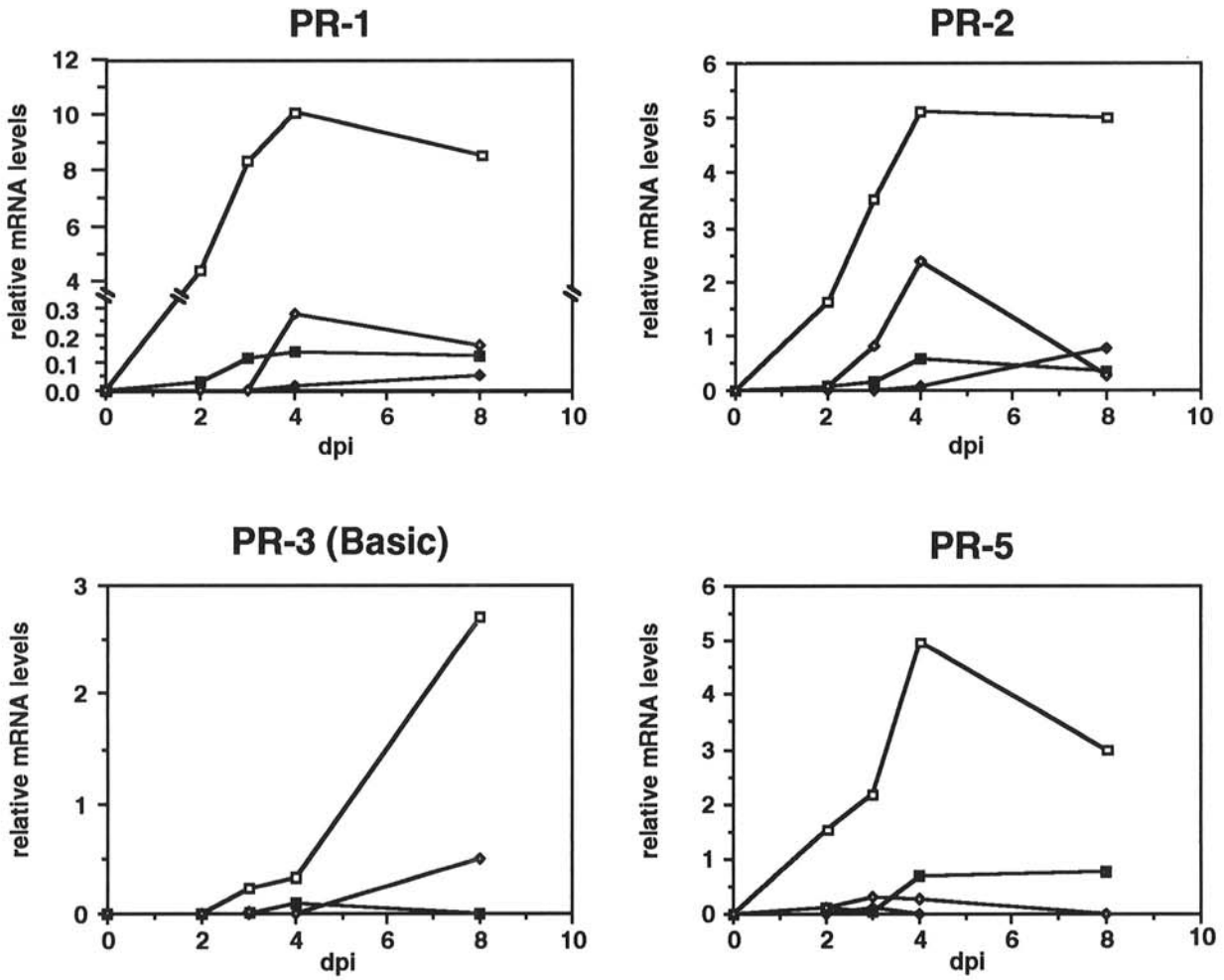


Fig. 5. Kinetics of pathogenisis-related (PR) mRNA PR-1, PR-2, PR-3 (basic), and PR-5 accumulation in inoculated and uninoculated leaves of turnip crinkle virus (TCV)-inoculated Di-17 and Di-3 plants. Autoradiograms of blots probed with the various PR gene clones (including the data shown in Fig. 4) were quantitated by normalizing the band intensity for each lane (determined by scanning autoradiographs with a Pharmacia LKB UltroScan XL, Pharmacia, Inc., Piscataway, NJ) with that of the ribosomal RNA control and subtracting the band intensity obtained at each time point for the corresponding leaves of mock-inoculated plants. Although the general trends of induction between the PR genes can be compared, the absolute values cannot be compared, due both to differences in scale and probe-specific activity. Di-17 inoculated leaves (□), Di-17 uninoculated leaves (■), Di-3 inoculated leaves (◇), and Di-3 uninoculated leaves (◆).

17 and Di-3 plants grown under normal light conditions exhibited very low to undetectable levels of PR-1, PR-2, or PR-5 mRNAs immediately after inoculation. In contrast, plants grown under continuous light showed elevated levels of messages for all of these genes. By 4 dpi, no induction of PR message could be observed in TCV-inoculated plants over that observed in the mock-inoculated plants (data not shown). These light conditions, therefore, were sufficient to induce a high basal level of PR gene expression that appears to correlate with increased resistance in both the resistant Di-17 and the susceptible Di-3 plants.

DISCUSSION

In this paper, we have characterized the interaction between TCV and two newly isolated lines of *A. thaliana* derived from the Dijon ecotype. In one line, Di-3, TCV rapidly replicated and spread throughout the plants, causing severe disease symptoms and death. This response was similar to that of other TCV-susceptible ecotypes, such as Columbia (20). Di-17 plants, unlike the previously reported Di-0 ecotype (35), consistently expressed a HR after viral inoculation, developing necrotic lesions on the inoculated leaves. The majority of Di-17 plants (~70%) showed no further symptoms, developing normally. Systemic symptoms ranging from mild to severe were observed in the remainder of the plants. We have recently discovered that the variability in resistance levels previously observed in our Di-17 line was primarily caused by slight differences in age at the time of inoculation due to asynchronous germination. We now see virtually 100% resistance in the vernalized Di-17 plants.

The discovery that Di-17 plants can exhibit 100% resistance to TCV infection increases the similarity between the *Arabidopsis*-TCV and the tobacco-TMV systems. In *Arabidopsis*, as in tobacco, susceptibility correlates with the lack of a HR and the rapid spread of virus throughout the plant. Additionally, the resistance response in *Arabidopsis*, like that of tobacco, is characterized by necrotic lesion formation on the inoculated leaves and viral restriction to these lesions. The inability to detect viral RNA elsewhere suggests that resistance to TCV is due to an ability to restrict viral movement and, therefore, is mediated at the whole plant rather than at the cellular level. In another ecotype, Leisner and Howell (19) have recently demonstrated that *Arabidopsis* also can resist cauliflower mosaic virus infection by preventing viral movement from the initial site of inoculation.

In contrast, the *Arabidopsis*-TCV system described by Simon et al (35) is quite different. The majority of the Di-0 plants do not develop necrotic lesions, and TCV is able to replicate throughout the inoculated leaf. In addition, Simon et al (35) have observed viral movement from the inoculated leaf to the opposite, uninoculated leaf. Currently, we cannot explain the discrepancies between our results.

The induction of the resistance response in TCV-inoculated *Arabidopsis*, similar to that of TMV-inoculated tobacco, was accompanied by the increased expression of several families of PR genes, including PR-1, PR-2, PR-3 (basic), and PR-5. These messages accumulated to substantial levels in the inoculated leaves. The acidic PR genes appeared to be coordinately regulated, analogous to the response documented in tobacco (40). The basic form of PR-3, however, accumulated with significantly different kinetics.

Messages for these same PR genes accumulated in the upper, uninoculated leaves at slightly later times and to lower levels. The correlation between increased PR gene expression in the uninoculated portion of the plant and the induction of acquired resistance has been established in a variety of systems, including TMV-inoculated tobacco (3,40). Moreover, treatment of *Arabidopsis* and tobacco with agents that induce acquired resis-

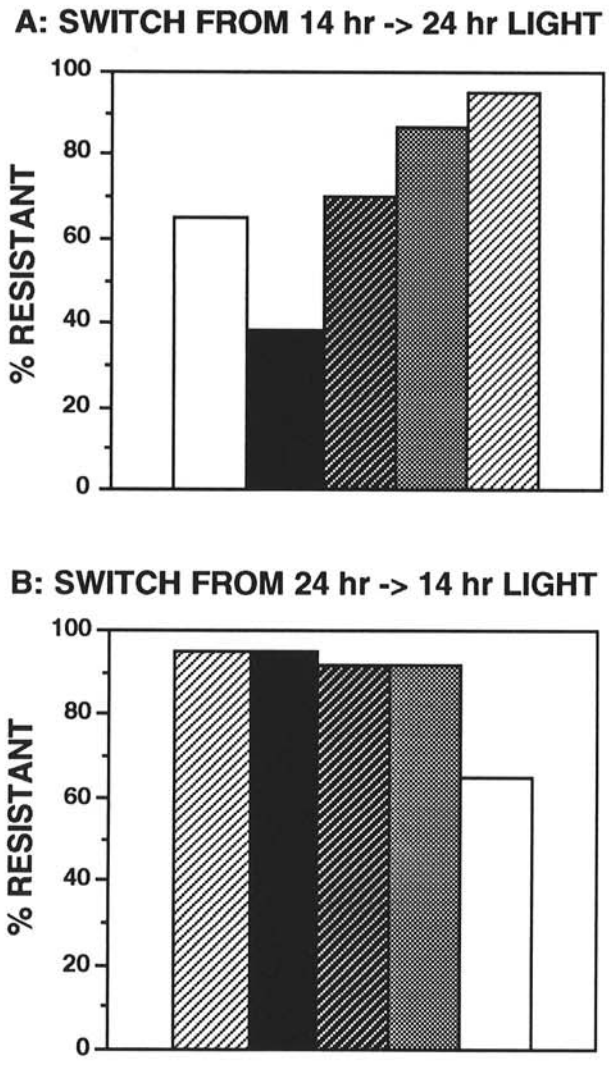


Fig. 6. The effect of light conditions on turnip crinkle virus (TCV)-resistance levels exhibited by Di-17. Di-17 plants were initially grown under **A,** normal light (14 h at 14,500 lx) or **B,** continuous light (24 h at 16,000 lx) conditions. At 4 days pre-inoculation (▩), 2 days pre-inoculation (▨), or at the time of inoculation (■) ~20 plants were switched to the alternate light condition. As a control, 20 plants also were retained at their original light conditions, either normal light (□) or continuous light (▨), after inoculation. The percentage of resistant plants was determined at 14 days post-inoculation.

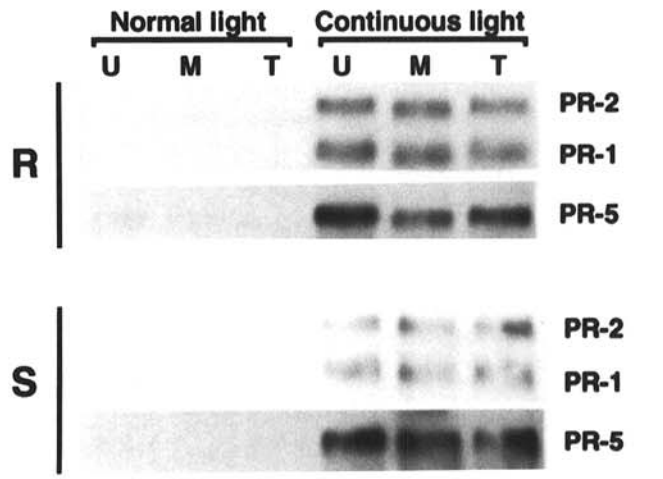


Fig. 7. Northern analysis of pathogenisis-related (PR)-1, PR-2, and PR-5 gene expression in Di-17 and Di-3 plants grown under normal and continuous light conditions. RNA was isolated from untreated (U), mock-inoculated (M), and turnip crinkle virus (TCV)-inoculated (T) Di-17 (R) and Di-3 (S) plants grown under normal or continuous light conditions immediately after inoculation. The blot, containing 5 μg of RNA per lane, was sequentially probed with PR-1, PR-2, and PR-5.

tance, such as 2,6-dichloroisonicotinic acid (INA), enhances the expression of the PR genes (39,40). Therefore, the PR gene induction observed in the uninoculated leaves of *Arabidopsis* reacting hypersensitively to TCV strongly suggests the presence of SAR. This is consistent with the observation that TCV-inoculated Dijon exhibits SAR to *Pseudomonas syringae* (S. Uknes, E. Ward, and J. Ryals, *personal communication*).

Susceptible Di-3 plants failed to exhibit a HR after TCV inoculation and accumulated significantly reduced message levels for the PR genes. In contrast, susceptible cultivars of tobacco do not express the PR genes after TMV inoculation. PR or PR-like gene expression has been documented, however, during the susceptible response of tobacco and tomato to a number of other viruses and viroids (4). In many of these cases, PR gene induction occurs at later times and to lower levels than in comparable resistant plants. Presumably this induction of defense-related genes is too late and/or weak to prevent pathogen replication and spread.

The interactions between *Arabidopsis* and a variety of other pathogens, including *Peronospora* (17) and pathovars of *Pseudomonas* and *Xanthomonas*, also have been described (10–12,36, 38,41). Inoculation of *Arabidopsis* with high concentrations of avirulent *Pseudomonas* leads to a HR, including inhibition of bacterial replication (10–12). Interestingly, the pattern of expression of defense-related genes during the resistance response to *Pseudomonas* differs from that observed after TCV inoculation. PR-2 gene expression in *Arabidopsis* inoculated with avirulent *Pseudomonas* does not correlate with the HR. Rather it is more highly expressed during the susceptible response (12). This finding suggests that in *Arabidopsis* the defense-related genes are induced differentially in response to different pathogens.

We and Simon et al (35) have observed that the level of resistance to TCV is significantly influenced by environmental conditions. When Di-3 and Di-17 plants were grown under continuous, high-intensity light, they exhibited unusual morphologies and elevated levels of resistance to TCV infection. In addition, we found that plants grown under high-intensity, continuous light constitutively expressed the PR-1, PR-2, and PR-5 genes at relatively high levels. Thus, increased PR gene expression again appears to correlate with enhanced resistance. However, it should be noted that there is no direct proof that PR proteins are involved in protection against viral infection (8). Moreover, although Di-3 and Di-17 plants grown under high-intensity, continuous light expressed similar levels of the PR genes, the Di-3 plants were eventually killed by the virus. This demonstrates that additional factors are required for resistance to viral infection.

The *Arabidopsis*-TCV system described in this paper provides many advantages for the further characterization of the resistance response. At present, only one plant gene controlling resistance has been isolated (15). The small genome and excellent genetics of *Arabidopsis* combined with the availability of closely related lines of TCV-resistant and -susceptible plants will greatly facilitate efforts to isolate such resistance genes, as well as the components of the signal transduction pathway leading to HR and SAR. The many similarities between the resistance response of *Arabidopsis* and that of other plants, including tobacco, to viral infection suggest that the *Arabidopsis*-TCV system will be a valuable tool for exploring this complex response.

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