The 5' Third of *Cauliflower mosaic virus* Gene VI Conditions Resistance Breakage in *Arabidopsis* Ecotype Tsu-0

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ABSTRACT

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Arabidopsis thaliana ecotypes vary in their responses to viruses. In this study, we analyzed the variation in response of *A. thaliana* ecotype Tsu-0 to *Cauliflower mosaic virus* (CaMV). This ecotype was previously reported to be resistant to two CaMV isolates (CM1841 and CM4-184), but susceptible to W260. In this study, we show that Tsu-0 is resistant to four additional CaMV isolates. CaMV propagated within the rosette leaves of Tsu-0 plants, but did not appear to spread systemically into the in-

florescence. However, virus viability in rosette leaves of Tsu-0 plants apparently was not compromised because infectious CaMV could be recovered from these organs. W260 overcomes Tsu-0 resistance by a passive mechanism (i.e., this virus avoids activating plant defenses). The portion of the viral genome responsible for W260 resistance breakage was mapped to the 5' third of gene VI, which we have termed RBR-1. This region is also responsible for controlling the ability of CaMV to infect different types of solanaceous plants. Hence, the pathways by which plants of different families interact with CaMV may be conserved through evolution.

Additional keywords: virus resistance.

In their native environments, plants are exposed to several types of pathogens (1). To respond to pathogens, plants possess resistance genes whose products control or prevent pathogen infection and disease development (19,42). Plant resistance gene products often inhibit specific steps in the life cycle of a pathogen (1,16). Interestingly, a resistance gene product may act by more than one pathway to protect the plant from a pathogen (6). In some cases, the viral genes involved in the resistance response have been determined through the discovery of resistance-breaking viruses and viral synergism (2,24–26,33,40). Viruses may overcome resistance by either an active or a passive mechanism. For example, the potyvirus helper component-proteinase (HC-Pro) gene product inhibits gene silencing (2,24). This permits resistance to be overcome because gene silencing is apparently a plant defense mechanism (45,47). Viruses can also overcome resistance by a passive process. For example, *Potato virus X* strain HB overcomes *Rx* gene-mediated resistance (27). This strain harbors a coat protein lacking a determinant needed to activate the *Rx* gene-mediated resistance pathway.

The cruciferous plant *Arabidopsis thaliana* has been useful for studying a variety of host–pathogen interactions (28). For example, the semidominant *Car1* gene of *A. thaliana* ecotype En-2 confers resistance to most isolates of *Cauliflower mosaic virus* (CaMV) (9,29,44). *Car1* inhibits the systemic spread of CaMV (30) and defense-related genes are activated in this plant upon virus infection (9). However, the En-2 ecotype is not resistant to CaMV isolate NY8153 nor to *Turnip mosaic virus* (44). These data indicate that resistance may be virus strain and isolate specific. However, the NY8153 gene or genes permitting the virus to overcome En-2 resistance are currently unknown.

CaMV is useful for studying virus–host interactions for several reasons, not the least of which is its ability to infect *A. thaliana* (29). The double-stranded, circular DNA genome of this plant pararetrovirus is packaged within particles that are 50 nm in diameter (22,31,37). The CaMV genome encodes seven to eight proteins. One of the more interesting proteins is that encoded by gene VI. Gene VI encodes a multifunctional protein involved in inclusion body formation, translation of viral genes, control of symptom expression, induction of the hypersensitive response (HR), and regulation of CaMV host range (3,8,13–15,17,26,35,36,39–42). Using chimeric CaMV genomes, we demonstrate that the 5' third of gene VI controls the capacity of the W260 isolate to overcome the resistance of *A. thaliana* ecotype Tsu-0 by a passive mechanism. Furthermore, this resistance appears to prevent the systemic spread of four additional CaMV isolates.

MATERIALS AND METHODS

***A. thaliana* ecotypes and virus inoculations.** *A. thaliana* ecotypes Tsu-0 and Col-0 used in this study were provided by S. H. Howell (Iowa State University, Ames). Seeds for these ecotypes were planted in moistened Redi Earth potting soil (BFG Supply, Burton, OH). Plants were propagated in a growth chamber under continuous light of 60 $\mu\text{E m}^{-2}\text{s}^{-1}$ of photosynthetic active radiation (PAR) at 20°C and fertilized with mineral nutrient solution as described previously (44).

Cloned viral DNA for CaMV isolate CM1841 (20) was provided by S. H. Howell. CaMV isolate W260 (21), as well as the chimeric viruses CaMVH30, CaMVH31, CaMVH33, CaMVH72, CaMVH82, and CaMVH84, were reported previously (40,46). CaMV isolates NY8153 (11), D/H (5), Cabb S (18), and CMV-1 (10) were provided by U. Melcher (Oklahoma State University, Stillwater). CaMV strain CaN (23) was provided by K. Richards (Institut de Biologie Moleculaire des Plantes du CNRS et de l'ULP, France). All CaMV isolates and chimeras were maintained in tur-

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nips (*Brassica campestris* L. var. *rapa* 'Just Right') by serial passage in a greenhouse at 22°C under natural lighting (29).

A. thaliana ecotypes Col-0 and Tsu-0 were inoculated at the six-leaf-stage with sap prepared from symptomatic turnip plants. Sap was prepared by harvesting turnip leaves infected with the appropriate virus and grinding in 3 ml of inoculation buffer (10 mM potassium acetate, pH 7.2) per gram of tissue. Celite was added and the suspension was mixed and rub-inoculated onto rosette leaves three, four, and five of *A. thaliana* plants with a plastic spatula (44). Following inoculation, *A. thaliana* plants were propagated in a growth chamber under a regime of 12 h of light (at 60 $\mu\text{E m}^{-2}\text{s}^{-1}$ PAR) and 12 h of dark at 20°C and fertilized with mineral nutrient solution as described previously (44). Plants were observed daily for symptom production up to 52 days postinoculation (DPI). Each experiment was performed at least two to three times with 15 to 20 plants of each ecotype inoculated per experiment. Although the inoculation efficiencies for this work appear somewhat low, they are typical for this type of experiment (29, 30,44).

Coinoculation experiments. *A. thaliana* ecotypes Tsu-0 and Col-0 were inoculated with plant sap from turnips infected with CaN or W260, or with a mixture of sap containing both viruses. The inoculum for single viral isolates was prepared as described above. Mixtures were prepared by grinding CaN- or W260-infected turnip tissue with 1 ml of inoculation buffer per gram of tissue to make a 3 $\times$ inoculum. The concentrated inoculum allowed the virus to be diluted threefold and still retain the same CaMV concentration as for a normal inoculation, as described previously. This permitted three different mixture concentrations to be prepared from these inocula: (i) 1 ml of sap from CaN-infected turnip tissue with 1 ml of W260 sap and 1 ml of inoculation buffer (1:1 mixture); (ii) 2 ml of CaN sap with 1 ml of W260 sap (2:1 mixture); and (iii) 1 ml of CaN sap with 2 ml of W260 sap (1:2 mixture). The viruses and mixtures were inoculated onto Col-0 and Tsu-0 plants as described previously (44). Three trials were conducted for each experiment with 15 to 20 plants per pot and two pots for each treatment.

Polymerase chain reaction analyses. The presence of viral DNA in infected plants was detected using polymerase chain reaction (PCR). The extraction procedure used to isolate and amplify viral DNA from infected plant tissue was derived from that described by Callaway et al. (9). All of the inoculated Tsu-0 and Col-0 plants from a pot (15 to 20 plants) were harvested by 52 DPI and separated into rosette leaf and cauline leaf/flower stalk tissue. The rosette leaves from all of the plants in each pot were pooled together. It is important to note that this pooled tissue included inoculated and uninoculated rosette leaves. Likewise, all of the cauline leaf/flower stalk tissue from all plants in each pot was pooled.

Tissue was ground in extraction buffer (250 mM NaCl, 0.25% sodium dodecyl sulfate, 30 mM EDTA, and 100 mM Tris, pH 8) at a concentration of 1 ml of buffer per gram of tissue. The extract was incubated with 10 μl of proteinase K (10 mg/ml) per ml of tissue extract at 55°C for 60 min. The extract was diluted with an equal volume of distilled water and incubated at 95 to 100°C for 8 to 10 min. The samples were permitted to cool, centrifuged at 13,000 $\times g$ for 5 min, and the supernatants were used as templates for PCR analyses.

PCR reactions were conducted in a 100- μl total volume that contained a final concentration of 50 mM KCl, 1.5 mM MgCl_2 , 10 mM Tris-HCl (pH 9.0), 0.1% Triton X-100, 200 μM for all four deoxynucleotide triphosphates, 0.5 units of *Taq* DNA polymerase (Promega Corporation, Madison, WI), and 2×10^{-7} M for the CM42F (5'-GGAAACAGTGCTTCATCCTC-3') and CM42R (5'-TCGCTGCAACGGCTTCTAAG-3') primers (synthesized by DNA International Inc., Lake Oswego, OR). To this mixture, either 10 μl of infected plant tissue extract or 10 ng of pCaMV10 (20), pCaMVW260 (21,40), or pCaN (23) DNA was added as a tem-

plate. The PCR program consisted of a five-cycle preamplification step of 30 s at 92°C, 90 s at 35°C, and 40 s at 72°C, followed by a single elongation step of 1 min at 95°C and 5 min at 72°C. Finally, a 35-cycle amplification step of 30 s at 94°C, 30 s at 45°C, and 40 s at 72°C was performed. The amplifications were performed in a TempTronic/Amplifon Programmable Thermal Cycler (Barnstead-Thermolyne, Dubuque, IA). The CM42F/CM42R primer pair directs the amplification of a 724-bp fragment-spanning gene II from all viruses and chimeras tested in this study, except for CaN. The CaN fragment is 430 bp in length. PCR products were fractionated by electrophoresis through a 1.5% agarose gel in 1 $\times$ Tris-borate EDTA buffer (4). Each set of reactions was tested at least twice for each set of inoculated plants.

RESULTS

***A. thaliana* ecotype Tsu-0 is resistant to CaMV.** *A. thaliana* ecotypes were previously reported to vary in their susceptibility to CaMV isolates (9,29). Ecotypes such as Col-0 are typically susceptible to CaMV, including CM1841 and W260 (29,30) (Table 1). Col-0 plants infected with CM1841 developed vein clearing and chlorosis on rosette and cauline leaves as well as chlorosis and stunting of the flower stalk (Fig. 1). Col-0 plants infected with W260 showed similar symptoms, but the flower stalks were usually more severely stunted. Symptoms in both cases appeared by 23 to 25 DPI.

As reported previously (29), Tsu-0 plants inoculated with CM1841 showed no symptoms (Fig. 1; Table 1), whereas those infected with the W260 isolate developed severe systemic symptoms (e.g., stunting and chlorosis of the rosette leaves). If the flower stalks and cauline leaves developed on W260-infected Tsu-0 plants, they were chlorotic and severely stunted, but these plants frequently died before they flowered ($\approx$ 52 DPI). In no case were necrotic lesions observed on leaves of Tsu-0 plants inoculated with either virus.

The initial evaluation of Tsu-0 plants involved only three isolates (29); therefore, it was important to extend these studies to other CaMV isolates. Thus, four additional CaMV isolates (Cabb S, CMV-1, D/H, and NY8153) were tested for their capacity to overcome resistance in Tsu-0 plants. All four isolates induced systemic infections in Col-0 (Table 1), showing the typical CaMV-induced chlorosis and stunting of the plants beginning 23 to 25 DPI. In contrast, none of the four isolates induced symptoms on either the inoculated or noninoculated portions of Tsu-0 plants.

CaMV resistance in Tsu-0 plants prevents invasion of inflorescence organs. To gain insight into Tsu-0 resistance, the distribution of CaMV DNA in plants inoculated with CM1841 and W260 was determined with PCR (9). Col-0 plants inoculated with

TABLE 1. Susceptibility of *Arabidopsis thaliana* ecotypes to systemic infection by *Cauliflower mosaic virus* isolates^a

Virus	Col-0	Tsu-0
Cabb S	18/50 (36)	0/53 (0)
CaN	12/54 (22)	0/50 (0)
CM1841	82/225 (36)	0/225 (0)
CMV-1	17/37 (46)	0/38 (0)
D/H	21/57 (37)	0/57 (0)
NY8153	17/73 (23)	0/75 (0)
W260	58/225 (26)	61/253 (24)
Mixtures ^b		
1:1	26/90 (29)	0/90 (0)
2:1	25/90 (28)	0/90 (0)
1:2	24/90 (27)	0/90 (0)

^a Number of plants showing symptoms by 52 days postinoculation out of the total number of plants analyzed. The percentage of infected plants is given in parentheses. The numbers are a summary of at least two experiments with 15 to 20 plants per experiment. Only two experiments were done for CMV-1; eight experiments were done for CM1841 and W260.

^b Indicates the ratio of CaN to W260 in coinoculations.

either virus, as well as noninoculated Tsu-0 and Col-0 plants, served as controls. CaMV DNA for both viruses was detected in both the rosette leaf and cauline leaf/flower stalk portions of W260- and CM1841-infected Col-0 plants (Fig. 2). It is important to note that the rosette tissue examined for all PCR experiments included both inoculated and uninoculated leaves. Viral DNA of both viruses also was detected in rosette leaf tissues for Tsu-0 plants. However, only W260 DNA was detected in the cauline leaf/flower stalk portions of Tsu-0 plants. These results were consistent for two separate experiments.

In addition to CM1841 and W260, PCR analyses were performed to determine the distribution of CaMV DNA in Col-0 and Tsu-0 plants inoculated with the Cabb S, CMV-1, D/H, or NY8153 CaMV isolates. Viral DNA of all four viral isolates was detected in both rosette leaf and cauline leaf/flower stalk portions of infected Col-0 plants (Fig. 2), but only in the rosette leaves of Tsu-0 plants. Identical results were obtained both times these experiments were performed.

To determine if CM1841 viability was compromised in Tsu-0 plants, 20 Tsu-0-inoculated plants within a pot were harvested and divided into rosette leaf and cauline leaf/flower stalk tissue; the same organs were pooled together. A pot of 20 CM1841-infected Col-0 plants also was harvested and analyzed in the same manner as a control. These tissues were ground in inoculation buffer and inoculated onto turnip plants as described previously. Turnips inoculated with sap derived from the rosette portions of CM1841-infected Tsu-0 plants developed chlorotic lesions and vein-clearing symptoms by 30 DPI. However, no symptoms were induced in turnips by 60 DPI, when they were inoculated with sap derived from the asymptomatic cauline leaf/flower stalk portions of CM1841-infected Tsu-0 plants. Turnips inoculated with sap prepared from either rosette leaf or cauline leaf/flower stalk tissue from Col-0 plants infected with CM1841 developed chlorotic lesions and vein-clearing symptoms. Turnips inoculated with tissue from either portion of Tsu-0 plants inoculated with W260 de-

veloped chlorotic lesions, vein-clearing, and stunting symptoms typical of that virus.

W260 overcomes Tsu-0 resistance via a passive mechanism.

To determine if W260 either actively or passively overcomes resistance, Tsu-0 and Col-0 plants were inoculated with mixtures of the W260 and the CaN isolates of CaMV. CaN was previously described by Jupin et al. (23) and consists of a CM4-184 CaMV genome in which gene II was replaced with the *N* gene from *Beet necrotic yellow vein virus*. The *N* gene insert for CaN is only 190 bp in length, whereas the wild-type gene II harbored by W260 is 479 bp. Thus, it is possible to detect DNA for both CaN and W260 in a mixture by PCR (Fig. 3).

Col-0 plants inoculated with either virus alone or with 1:1, 1:2, or 2:1 mixtures of CaN/W260 developed systemic symptoms (Table 1), including chlorosis and vein clearing on the rosette and cauline leaves and stunting of the flower stalk. The symptoms induced by CaN on Col-0 were identical to those incited by CM1841 as well as its CM4-184 progenitor (data not shown). In contrast, none of the Tsu-0 plants inoculated with either of the three mixtures, or with CaN alone, developed symptoms. Only Tsu-0 plants inoculated with W260 alone developed the typical chlorotic lesion, vein-clearing, and stunting symptoms.

PCR analyses performed on tissue from Col-0 plants infected with either CaN or W260 alone revealed viral DNA within rosette leaves as well as in cauline leaf/flower stalk tissues (Fig. 3). Col-0 plants inoculated with 1:1, 1:2, or 2:1 ratios of W260 to CaN also were infected by both viruses in rosette leaf and in cauline leaf/flower stalk tissues (only the 1:1 mixture is shown in Fig. 3). These data indicated that there was no interference between the W260 and CaN isolates in the infection of susceptible Col-0 plants.

PCR analysis of plant tissue from Tsu-0 plants showed a different pattern. W260 DNA was detected in the rosette leaves and in the cauline leaf/flower stalk portions of infected Tsu-0 plants (Fig. 3). However, PCR analyses of Tsu-0 plants inoculated with

Fig. 1. Infectivity of *Cauliflower mosaic virus* in *Arabidopsis thaliana* ecotypes Tsu-0 and Col-0. Col-0 and Tsu-0 plants were either mock inoculated or inoculated with either CM1841 or W260 and photographed 52 days postinoculation.

CaN or with either of the three mixtures revealed viral DNA within the rosette leaf but not in the cauline leaf/flower stalk portions of these plants. Similar results obtained for two independent experiments.

The 5' third of the gene VI region of CaMV isolate W260 allows for breakeage of Tsu-0 resistance. W260 overcomes Tsu-0 resistance but CM1841 does not; therefore, it is possible to map the portion of the viral genome responsible for resistance breakeage by using viral chimeras. Genetic chimeras have been used previously to identify several functions within the CaMV genome (35, 40,41,43). Therefore, this approach was used to examine Tsu-0 resistance breakeage. The initial chimeras used in this study were viruses in which a region spanning the majority of gene VI and the large intergenic region was exchanged between W260 and CM1841 (Fig. 4) (40). By 23 to 25 DPI, both H30 and H31 induced systemic symptoms on Col-0 that were typical of CaMV on *A. thaliana* (i.e., chlorosis and vein clearing of leaves, chlorosis and stunting of the flower stalk). The situation with the Tsu-0 ecotype was quite different. The H30 virus (W260 + CM1841 gene VI) was unable to induce either local or systemic symptoms on Tsu-0 plants, whereas the H31 chimera (CM1841 + W260 gene VI) induced systemic symptoms on Tsu-0 plants, including chlorosis and vein-clearing symptoms on leaves, and stunting of the flower stalk by 23 to 25 DPI. These data implicated gene VI, the adjacent intergenic region, or both as containing the determinant conferring the resistance-breaking ability on CaMV isolate W260.

To further localize the resistance-breaking determinant, the H84 and H82 chimeras (46) were employed. These two chimeras con-

sist of W260 genomes in which large portions of gene VI and the intergenic region were replaced with the equivalent segment from CM1841 (Fig. 4). Both chimeras were able to systemically infect Tsu-0 and Col-0 plants, inducing symptoms that were typical of CaMV infection in *A. thaliana*. The results obtained with these two chimeras suggested that the 5' third of gene VI harbored the resistance-breaking determinant. To examine this hypothesis, two additional chimeras were tested, H72 and H33 (46). Whereas both H72 and H33 induced systemic infections in Col-0 that showed the typical CaMV symptoms, only the latter was able to systemically infect Tsu-0 plants.

To determine the distribution of the viral chimeras in Col-0 and Tsu-0 plants, PCR analyses were performed (Fig. 5). The PCR data agreed with the visible symptom data and were consistent for two independent experiments. CaMV DNA was detected in both the rosette leaf and the cauline leaf/flower stalk portions of Col-0 plants inoculated with all of the chimeras. Viral DNA also was detected in the rosette leaf portions of Tsu-0 plants inoculated with all of the chimeras. However, CaMV DNA was only detected in the cauline leaf/flower stalk tissues of Tsu-0 plants inoculated with H31, H84, H82, and H33, but not with H72 and H30.

DISCUSSION

A. thaliana ecotype Tsu-0 was previously reported to be resistant to CaMV isolates CM1841 and CM4-184, but susceptible to W260 (29). The work described here demonstrates that Tsu-0 resistance is active against four additional CaMV isolates. Previ-

Fig. 2. Polymerase chain reaction (PCR) detection of *Cauliflower mosaic virus* (CaMV) in *Arabidopsis thaliana* ecotypes Tsu-0 and Col-0. Tsu-0 or Col-0 plants, either mock inoculated or inoculated with several *Cauliflower mosaic virus* isolates, were harvested 52 days postinoculation and analyzed by PCR. O = mock-inoculated plant. CaMV isolates: W260, W; NY8153, N; D/H, D; CM1841, M; CMV-1, V; and Cabb S, A. K indicates the New England Biolabs (Beverly, MA) 100-bp ladder. U indicates samples from cauline leaves/flower stalks; L indicates samples from rosette leaf tissue.

Fig. 3. Infectivity of *Cauliflower mosaic virus* isolates W260, CaN, or mixtures of W260 and CaN in *Arabidopsis thaliana* ecotypes Tsu-0 or Col-0. Tsu-0 or Col-0 plants inoculated with either CaN, C; W260, W; or a 1:1 mixture of the two viruses, M. Plant tissues were harvested at 52 days postinoculation and analyzed by polymerase chain reaction (PCR). O indicates mock-inoculated Tsu-0 plant; D indicates PCR product generated from plasmid DNA. U indicates PCR product generated from cauline leaf/flower stalk portions of the plants and L from rosette leaf tissue. Arrow shows location of the 724-bp W260 PCR fragment; arrowhead indicates the 430-bp CaN product.

ous work (29) did not elucidate the nature of Tsu-0 resistance to CaMV. To examine this, PCR analyses were performed on Tsu-0 plants inoculated with the CaMV isolates. We discovered that virus DNA was present in the rosette leaves but absent elsewhere in CM1841-inoculated Tsu-0 plants. The same was true of Tsu-0 plants infected with CaMV isolates Cabb S, CMV-1, D/H, and NY8153. Analysis of CM1841-infected Tsu-0 plants by the plant skeleton hybridization method (30,32), suggests that this virus not only is limited to the rosette leaves but is localized to the inoculated leaf (data not shown). Hence, Tsu-0 appears to prevent invasion of the inflorescence by several CaMV isolates. Based on visible symptom data, Tsu-0 was resistant to several other CaMV isolates, including: Bari-1, BBC, Cabb B, and California (data not shown). It is likely that these viruses are also prevented from invading the inflorescence; therefore, this indicates that Tsu-0 is resistant to systemic infection by most CaMV isolates. This suggests that CaMV isolate W260 may be unique in its interaction with Tsu-0 plants.

The inability of NY8153 to systemically infect Tsu-0 plants is interesting because this virus isolate is able to overcome resistance in another *A. thaliana* ecotype, En-2 (44). We have shown here that gene VI from CaMV isolate W260 is necessary and sufficient for CM1841 to overcome Tsu-0 resistance. However, the viral genetic basis of NY8153 resistance breakage in En-2 plants appears to be more complex: NY8153 gene VI is not sufficient to confer resistance-breaking capacity to isolates unable to systemically infect En-2 plants (44). These data may indicate that *A. thaliana* ecotypes harbor resistance genes that place different requirements on CaMV isolates.

Fig. 4. Genetic map of *Cauliflower mosaic virus* (CaMV) chimeras. Linearized versions of the CaMV genomic map are shown (40,46). Roman numerals indicate the locations of the various CaMV genes. The 5' to 3' coding of each gene is from left to right. The CM1841 (unshaded) and W260 (black) viral genomes are indicated. The number of plants showing systemic symptoms out of the total number of plants inoculated is shown. These numbers are from at least three experiments with 15 to 30 plants per experiment. ^a Numerical data are given in Table 1.

However the resistance shown by Tsu-0 is mediated, it apparently does not involve the HR because none of the CaMV isolates inoculated to that plant induced necrotic symptoms. Viable virus could be obtained from the rosette leaf portion of Tsu-0 plants inoculated with CM1841 but not from the cauline leaf/flower stalk tissues. These data suggest that CM1841 is not completely eliminated from inoculated leaves. In addition, the lack of viable virus in the cauline leaf/flower stalk tissues suggests that the virus does not systemically invade the inflorescence.

In contrast to the other CaMV isolates, W260 was able to systemically infect Tsu-0 plants. The W260 virus propagated and spread within the Tsu-0 ecotype so efficiently that it often killed infected plants before they flowered. We do not believe that the difference between CM1841 and W260 on Tsu-0 is due to a difference in inoculum titer, because (i) we standardized the inoculum concentration by adding the same amount of buffer per gram of tissue and (ii) the virus levels per gram of infected turnip tissue (used for *A. thaliana* inoculum) are higher for CM1841 than for W260 (3). Taken together, this means that there should be sufficient CM1841 inoculum to cause a systemic infection in Tsu-0 plants if infection of Tsu-0 were possible. Hence, it seemed more likely that there was a fundamental genetic difference between viruses such as CM1841 and W260 that permitted the latter to overcome Tsu-0 resistance.

To identify the portion of the viral genome responsible for this difference, we utilized viral chimeras constructed from CM1841 and W260. Analysis of these chimeras initially implicated gene VI as responsible for CaMV to infect Tsu-0 plants. Analysis of additional chimeras localized the resistance-breaking determinant to the 5' third of gene VI, which we have termed resistance-breaking region 1 (RBR-1). RBR-1 differs at 12 positions in nucleotide sequence between W260 and CM1841 (46). Notably, the 3' portion of RBR-1 overlaps with the sequence of gene VI encoding the minimal translational transactivation domain (17). Therefore, it is possible that the translational transactivation efficiencies of the W260 and CM1841 gene VI products may differ, permitting W260 to overcome Tsu-0 resistance.

Gene VI has been shown to be a factor influencing CaMV long-distance movement and resistance responses in solanaceous plants (38–41). For example, CaMV isolate CM1841 elicits a resistance response in *Nicotiana bigelovii*, whereas W260 spreads systemically through this host (40). It is the RBR-1 region of gene VI that also controls resistance breakage in *N. bigelovii* (46). This may imply that the plant factors influencing the role of gene VI in systemic spread or resistance-breakage may be conserved between the *Brassicaceae* and the *Solanaceae*.

Resistance shown by Tsu-0 plants could be overcome by either an active or passive process mediated by the W260 gene VI product. To address these possibilities, coinoculation experiments were

Fig. 5. Mapping of the domain of *Cauliflower mosaic virus* (CaMV) gene VI involved in overcoming resistance in *Arabidopsis thaliana* ecotype Tsu-0. Tsu-0 or Col-0 plants, either mock inoculated or inoculated with several CaMV chimeras, were harvested 52 days postinoculation and analyzed by polymerase chain reaction (PCR). K indicates the New England Biolabs (Beverly, MA) 100-bp ladder; O indicates mock-inoculated plant, either Tsu-0 or Col-0. L indicates PCR product generated from rosette leaf tissue and U from cauline leaf/flower stalk portions of the plants.

performed. These experiments suggest that W260 overcomes Tsu-0 resistance by evading the host recognition machinery, whereas other CaMV isolates, such as CM1841, appear to be recognized. When CaN and W260 were coinoculated onto Tsu-0 plants, the resistance pathway elicited by CaN apparently protected the plant against W260. These data suggest that gene VI does not act as a gene silencing suppressor like the potyvirus *HC-Pro* gene (2, 24). Rather, W260 infection of Tsu-0 more closely resembles the situation with the capsid protein of *Potato virus X* strain HB and its interactions with the potato *Rx* gene (7,27). The *Rx* gene-mediated resistance appears to be an induced response; therefore, it is possible that Tsu-0 resistance may be generated via a similar pathway. This response is probably mediated by a signal because it is unlikely that all of the cells infected with W260 in Tsu-0 plants also were infected with CaN.

Our work indicates that gene VI of CaMV isolates such as CM1841 and CaN induces a resistance response in Tsu-0 plants. Interestingly, gene VI can act as an avirulence gene in *Nicotiana* spp. Experiments with chimeric CaMVs or agroinfiltration experiments with gene VI indicate that this viral protein induces an HR in *N. edwardsonii* as well as production of pathogenesis-related proteins (34). However, not all resistance to CaMV in *Nicotiana* spp. is mediated via the HR. It is possible to separate the resistance in *N. edwardsonii* into two components, each controlled by a specific gene: a cell death component and a defense response independent of the HR (12). It is possible that Tsu-0 resistance operates via the second mechanism (i.e., a defense response that does not involve the HR). It is significant that the W260 RBR-1 region that permits Tsu-0 resistance to be overcome also elicits a cell death response in *N. clevelandii* (26). The *N. clevelandii* HR appears to be conditioned by a single recessive host gene within the plant. Taken together, these data indicate that the W260 RBR-1 region does not allow for evasion of host defenses in all plant species.

In summary, Tsu-0 resistance is mediated by prevention of CaMV systemic invasion of the inflorescence. This resistance is overcome by W260, which apparently is not recognized by the Tsu-0 host. The region of W260 responsible for overcoming resistance is the 5' third of gene VI, which we have termed RBR-1. The identification of RBR-1 will now permit studies to identify the precise genetic determinants responsible for overcoming Tsu-0 resistance.

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