Cauliflower Mosaic Virus Isolate NY8153 Breaks Resistance in *Arabidopsis* Ecotype En-2

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ABSTRACT

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Arabidopsis thaliana ecotype En-2 was previously shown to be resistant to cauliflower mosaic caulimovirus (CaMV) isolate CM4-184. In this study, En-2 plants were screened with eight other isolates of CaMV to identify viruses capable of overcoming resistance and to determine if the mechanism of resistance was the same for each virus. En-2 resistance to most CaMV isolates was mediated by the same mechanism, i.e., pre-

venting virus long-distance movement. One CaMV isolate, NY8153, was found that produced a severe systemic infection on En-2 plants. In addition, the CM1841 isolate was able to spread systemically through En-2 plants, to a limited extent, without producing visible symptoms. These data indicate that the resistance shown by En-2 plants is not an all-or-none phenomenon. En-2 plants were susceptible to turnip mosaic potyvirus, suggesting that resistance is specific to CaMV.

Additional keywords: long-distance virus movement, resistance breakage, virus resistance.

Plant viruses need to overcome many obstacles to produce disease (36). They must enter wounded cells, uncoat, replicate, express their genes, move from cell to cell within the host, and move long distance through the plant vascular system. Due to the complexity of the infection process, resistance results when any one of these steps is prevented (11,36). Virus resistance is often mediated by plant genes that block specific steps in the viral life cycle. For example, the *Nicotiana glutinosa* *N* gene and the tomato *Tm-1* gene block viral replication, whereas the tomato *Tm-2* gene inhibits the cell-to-cell movement of tobamoviruses (12,40,41,44,55). In addition to the resistance genes mentioned above, plant genes that inhibit the systemic spread of specific viruses have been identified (5,19,28,33).

To understand virus resistance, it is necessary to examine this problem from the perspective of both the host and the pathogen. *Arabidopsis thaliana* is a powerful tool for studying virus resistance, because the genetics and molecular biology of this cruciferous plant make it possible to identify and isolate the host genes involved (29). In *A. thaliana*, as in other plants, resistance genes inhibit specific steps in the viral life cycle. For example, *A. thaliana* ecotype En-2 harbors a single semidominant gene called *CAR1* that confers resistance to cauliflower mosaic caulimovirus (CaMV) (5,33). En-2 plants do not react to CaMV infection by producing a hypersensitive response, although infected plants do express certain defense genes (5). Because plants bearing the other resistance genes mentioned above produce a hypersensitive response, it is likely that the *CAR1* gene employs a different resistance pathway. To further emphasize this point, plants bearing the other resistance genes inhibit viral propagation in inoculated leaves, whereas plants harboring *CAR1* inhibit the long-distance movement of CaMV (5,33).

Virus long-distance movement, or spread of virus through the plant vascular system, is a complex process. The plant vascular tis-

sue through which most viruses move long distance is phloem tissue. To invade, move through, and exit the phloem, a virus must cross several different types of cellular boundaries (6,17,31,34, 53). It is known that plant viruses use plasmodesmatal connections to move from cell to cell within an infected leaf (6,34). However, the plasmodesmata at various locations within the long-distance movement pathway are structurally and functionally different from those mediating normal cell-to-cell movement (6,17,31,34). The fact that certain plant resistance genes permit cell-to-cell movement, but impair long-distance movement, also indicates that the host functions required for these two processes are different (6,34, 48). Because they must cross several types of boundaries to enter the phloem, viruses such as CaMV require a certain amount of time to invade the plant vascular system (32,33).

Once within conducting cells of the phloem, viruses travel with the flow of photoassimilates (the exported products of photosynthesis) from source (net photoassimilate exporters) to sink (net photoassimilate importers) tissues (31). Source organs such as mature leaves are incapable of importing viruses, whereas young sink leaves do import viruses. As a plant matures, sink leaves develop into source leaves. Because the kinetics of virus movement closely matches the rate of plant development, the more rapidly a plant matures, the smaller the number of leaves that a virus can invade. Such a plant is said to show developmental resistance (31,33).

As one might expect, several viral genes play an essential role in long-distance movement. Several plant viruses require coat protein to spread by the long-distance movement pathway, even though they can spread from cell to cell in its absence (6,17,31,34, 53). For example, coat protein or virion assembly mutants of tobacco mosaic virus (TMV) are unable to spread by the long-distance movement pathway, even though they can spread to the phloem by cell-to-cell movement (13). In addition to the coat protein gene, the TMV replicase gene also plays a role in long-distance movement (42). Thus, more than one gene within a viral genome may condition the ability of a virus to move long distance. Certain viral genes encoding accessory functions have also been shown to be determinants of virus long-distance movement. For example, the helper component proteinase gene of tobacco etch potyvirus functions in virus long-distance movement (9).

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CaMV is a pararetrovirus (26,36,47), and one of the seven genes encoded by this virus, gene VI, has been implicated as a factor controlling long-distance movement (45,49,50). However, it is possible that other viral genes, in addition to gene VI, play a role in CaMV long-distance movement. To identify viral genes involved in long-distance movement, we chose to take advantage of virus resistance. En-2 plants prevent CaMV long-distance movement (5,33). Therefore, if CaMV isolates capable of breaking En-2 resistance could be found, then they would be useful for identifying the viral genes involved in long-distance movement. To identify viruses capable of overcoming resistance, *A. thaliana* ecotypes En-2 and Col-0 were screened with different CaMV isolates. The mechanism of En-2 resistance was then examined in greater detail with the plant skeleton hybridization technique. Finally, the specificity of resistance shown by the En-2 ecotype was tested. En-2 resistance was not absolute, since one viral isolate was found to break resistance and another was able to spread systemically through these plants to a limited extent. For the other viral isolates tested, the mechanism of resistance appeared to be the same as with CM4-184, i.e., the inhibition of long-distance movement. However, virus resistance in En-2 plants was found to be specific to CaMV.

MATERIALS AND METHODS

Ecotypes used. The *A. thaliana* Col-0 and En-2 ecotypes used in this study were obtained from R. Last (Boyce Thompson Institute for Plant Research, Ithaca, NY). Seeds were planted in moistened Magic Moss potting soil (PVP Industries, Inc., North Bloomfield, OH) and vernalized for 2 weeks at 4°C in 4-inch-diameter fiber pots covered with plastic wrap. The plants were then grown in a light room under continuous illumination at 60 $\mu\text{E m}^{-2} \text{ s}^{-1}$ of photosynthetically active radiation (PAR) at 20°C. Three days after the seeds germinated, the plastic wrap was removed. Plants were subirrigated by soaking pots in a plastic flat. Plants were watered twice a week, once with tap distilled water and once with mineral nutrient solution (15). The plants were watered with tap distilled water for 4 h. Three days later, they were watered with mineral nutrient solution overnight. Fourteen days after germination, the plants were thinned to 20 plants per pot. The plants were then mechanically inoculated with virus 10 days after thinning. At this stage, plants usually had four to five rosette leaves.

Viral isolates, inoculations, and growth conditions. Virus-infected plant leaves and the cloned genomes for CM4-184 (23,24) and CM1841 (16) were a gift from S. Howell (Boyce Thompson Institute for Plant Research). The cloned genomes for CaMV isolates D/H (2) and BBC (7) were a gift from U. Melcher (Oklahoma State University, Stillwater). Virus-infected plant leaves and the cloned genomes for NY8153 (8), Cabb B (25), D4 (52), and California were a gift from J. Schoelz (University of Missouri, Columbia). Turnip leaves infected with CaMV isolate Harris were also provided by J. Schoelz.

All CaMV isolates used in this study were maintained by serial passage in *Brassica campestris* L. var. *rapa* "Just Right" (turnips) in a growth chamber at 20°C under continuous illumination of 60 $\mu\text{E m}^{-2} \text{ s}^{-1}$. For each viral isolate, 20 *A. thaliana* plants were mechanically inoculated with cell sap prepared by grinding infected turnip leaves in 10 mM potassium acetate buffer (pH 7.2) at 3 ml of buffer per gram of tissue. Celite was added at 6 mg ml⁻¹ of cell sap, and 5 μl of suspension was rubbed on each of three leaves (leaves 2, 3, and 4) per plant with a plastic spatula. Leaves were rinsed with water 5 min after inoculation, and inoculated plants were incubated for 8 h at room temperature under room lights. Plants were maintained in a growth chamber under a cycle of 12 h of light and 12 h of dark at 20°C. The total photosynthetic active radiation of about 60 $\mu\text{E m}^{-2} \text{ s}^{-1}$ of PAR was provided by fluorescent lamps. Plants were observed every day for systemic symptoms and finally assessed for symptoms at 52 days postinoculation (DPI). Each experiment was performed three times.

Turnip mosaic potyvirus (TuMV) strain Escarole 8 was a gift from R. Provvidenti (Cornell Agricultural Experimental Station, Geneva, NY) and was propagated in turnips. Turnip and *A. thaliana* plants were mechanically inoculated with TuMV as described above for CaMV. The inoculation of *Arabidopsis* plants with TuMV was performed twice.

Plant skeleton hybridization. The 8-kb viral genomes for seven of the nine different CaMV isolates were excised from their plasmid vectors by cleavage with *SaI*I restriction enzyme (Promega Corp., Madison, WI) using the buffers provided by the manufacturer. The genome of Cabb B was excised from its plasmid vector by cleavage with *SsI*I restriction enzyme (Gibco-BRL, Gaithersburg, MD) using the buffers provided by the manufacturer. The

TABLE 1. Visible symptoms and distribution of virus DNA in *Arabidopsis* ecotypes En-2 and Col-0 inoculated with nine isolates of cauliflower mosaic caulimovirus by 52 days postinoculation

Virus	Det ^a	Col-0				En-2			
		I ^b	R	C	FS	I	R	C	FS
BBC	V	0/20 ^c	8/20	8/20	8/20	0/20	0/20	0/20	0/20
	P	8/20	8/20	8/20	8/20	5/20	0/20	0/20	0/20
Cabb B	V	0/20	6/20	6/20	6/20	0/20	0/20	0/20	0/20
	P	6/20	6/20	6/20	6/20	4/20	0/20	0/20	0/20
California	V	0/20	6/20	6/20	6/20	0/20	0/20	0/20	0/20
	P	6/20	6/20	6/20	6/20	4/20	0/20	0/20	0/20
CM1841	V	0/20	11/20	11/20	11/20	0/20	0/20	0/20	0/20
	P	11/20	11/20	11/20	11/20	4/20	0/20	0/20	4/20
CM4-184	V	0/20	4/20	4/20	4/20	0/20	0/20	0/20	0/20
	P	4/20	4/20	4/20	4/20	2/20	0/20	0/20	0/20
D4	V	0/20	0/20	0/20	0/20	0/20	0/20	0/20	0/20
	P	0/20	0/20	0/20	0/20	0/20	0/20	0/20	0/20
D/H	V	0/20	8/20	8/20	8/20	0/20	0/20	0/20	0/20
	P	8/20	8/20	8/20	8/20	4/20	0/20	0/20	0/20
Harris	V	10/20	10/20	10/20	10/20	0/20	0/20	0/20	0/20
	P	10/20	10/20	10/20	10/20	4/20	0/20	0/20	0/20
NY8153	V	0/20	9/20	9/20	9/20	0/20	2/20	2/20	2/20
	P	9/20	9/20	9/20	9/20	4/20	2/20	4/20	4/20

^a Det = method for determining the presence of the virus: V = visible symptoms and P = plant skeleton hybridization.

^b Plant organs examined for presence of virus: I = inoculated leaves, R = noninoculated rosette leaves, C = cauline leaves, and FS = flower stalk.

^c Number of plants showing either visible symptoms or viral DNA within a plant organ out of total plants analyzed. These numbers are an average from three separate experiments for both plant skeleton and symptom data.

CM1841 genome was also used to detect the distribution of viral DNA of the Harris isolate, because the genome of this isolate was not available in a cloned form and Harris DNA cross-hybridized to CM1841.

After restriction enzyme cleavage, the virus genome and vector bands were separated on a 0.8% Agarose NA gel (Pharmacia LKB Biotechnology Inc., Piscataway, NJ) using 1× Tris-borate-EDTA buffer (1). Following electrophoresis, the bands corresponding to the virus genomes were excised from the gel, placed into 0.5-ml Eppendorf tubes with a hole in the bottom (made with an 18-gauge needle), and the tubes packed with glass wool. The 0.5-ml tube with the gel slice was then placed into a 1.5-ml Eppendorf tube that was, in turn, placed into a microcentrifuge (Fisher Scientific Co., Pittsburgh) and centrifuged at 6,000 rpm ($5,000 \times g$) for 5 min. The solution that collected in the 1.5-ml tube was then phenol-extracted and ethanol-precipitated (1). The precipitated viral genomic DNA was collected by centrifugation at top speed for 10 min in a microcentrifuge, the supernatant decanted, and the DNA pellet dried in a Centrivap (Labconco, Kansas City, MO). The dried DNA pellet was then dissolved in 20 μ l of 0.1× Tris-EDTA buffer (1 mM Tris and 0.1 mM EDTA, pH 7.5).

The viral genomes were labeled with a Multiprime DNA Labeling Kit (Amersham Corp., Arlington Heights, IL) according to manufacturer's instructions and used as probes. *A. thaliana* plants were harvested at 52 DPI and subjected to plant skeleton hybridization analysis as described by Melcher et al. (39) and modified by Leisner et al. (32,33). The complete cloned genome of the respective viral isolate was used as a probe. Each plant skeleton hybridization experiment was performed three times.

RESULTS

Identification of CaMV isolates overcoming En-2 resistance.

A. thaliana ecotype En-2 was resistant to three different CaMV isolates, whereas the Col-0 ecotype was susceptible to all three viruses (30). To identify viruses capable of overcoming resistance, En-2 ecotype plants were inoculated with nine different CaMV isolates (Table 1). Col-0 plants were inoculated with these viruses as a control. As expected, most CaMV isolates produced visible systemic symptoms on *A. thaliana* Col-0 plants. Only D4 failed to produce visible symptoms on this ecotype by 52 DPI. Systemic symptoms observed were the formation of chlorotic lesions and vein clearing on leaves, and chlorosis of the flower stalk (30,33,37). Systemic symptoms were observed on Col-0 when inoculated with every virus isolate except D4. However, symptoms were observed on the inoculated leaves of only those plants inoculated with CaMV isolate Harris. The other CaMV isolates were asymptomatic on inoculated leaves.

In contrast to the situation with Col-0 plants, most CaMV isolates did not induce visible symptoms on the resistant En-2 eco-

Fig. 1. Cauliflower mosaic caulimovirus isolate NY8153 produces a severe systemic infection on En-2 plants. En-2 plants were either **A**, mock-inoculated or **B**, inoculated with NY8153 24 days following germination and photographed at 45 days postinoculation.

Fig. 2. Distribution of NY8153 virus in En-2 plants. En-2 plants were either **A**, mock-inoculated or **B and C**, inoculated with NY8153, harvested at 52 days postinoculation, and analyzed by the plant skeleton hybridization technique. **B**, Asymptomatic En-2 plant and **C**, symptomatic En-2 plant. Arrows indicate inoculated leaves. Arrowheads in **B** indicate cauline leaves containing virus DNA; arrowhead in **C** indicates flower stalk. Note, the plant in **B** had two inoculated leaves remaining at the time of harvest.

type plants (Table 1). However, one CaMV isolate, NY8153, did induce visible symptoms on En-2 plants (Fig. 1). The rosette leaves, cauline leaves, and the flower stalk were extremely stunted in the En-2 plants inoculated with NY8153; however, the plants showed chlorosis on only a few of the cauline leaves. At about 47 DPI, most of the chlorotic material died, and the plants became extremely stunted and dark green in color. This is in marked contrast to the symptoms induced by this virus on Col-0, in which stunting, extensive chlorosis, and eventual death by 58 DPI were observed. Polymerase chain reaction (PCR) analysis of the cauline leaves showed that CaMV was indeed present within the symptomatic organs of NY8153-infected En-2 plants (data not shown). In addition, tissue homogenates from symptomatic En-2 tissue induced a systemic infection when inoculated to turnip plants.

Mechanisms of resistance for the En-2 ecotype plants. The lack of visible symptoms induced by the resistant En-2 ecotype in response to most CaMV isolates could be due to the inability of the virus to systemically infect the plants (33). Alternatively, these plants could be tolerant to CaMV, i.e., a symptomless systemic infection is induced (11). To examine the mechanism of En-2 resistance in greater detail, plant skeleton hybridization analyses were performed. Plant skeleton hybridization analysis was used to identify the step in the infection cycle of CaMV isolate CM4-184 blocked in En-2 plants (30,33). This technique showed that, whereas the virus was able to propagate within the inoculated leaves, long-distance movement of CaMV isolate CM4-184 was inhibited in En-2 plants. It was unknown whether resistance to other CaMV isolates was mediated by the same mechanism. Therefore, plant skeleton hybridization analyses were performed on En-2 plants infected with CM4-184 and the eight other CaMV isolates to examine the mechanism of resistance to these viruses.

Col-0 plants inoculated with each of the nine CaMV isolates were analyzed by the plant skeleton hybridization technique and served as positive controls. In Col-0 plants, the plant skeleton results reflected the symptom data (Table 1). As expected, viral DNA was not detected in D4-inoculated Col-0 plants, even in the inoculated leaves. This was intriguing, because the D4 isolate can induce a systemic infection on other *Arabidopsis* ecotypes (data not shown). Virus DNA for all isolates except D4 was detected in the inoculated leaves of Col-0 plants, even though the Harris isolate was the only CaMV isolate that induced symptoms in these leaves.

In En-2 plants, viral DNAs for six of the nine CaMV isolates (BBC, Cabb-B, California, CM4-184, D/H, and Harris) were detected in the inoculated leaves, but nowhere else within the plants. This indicates that these viral isolates can propagate in the inoculated leaves, but they lack the ability to infect En-2 plants systemically. However, fewer En-2 plants showed hybridization in inoculated leaves than did Col-0 plants. These data indicate that En-2 is less susceptible to CaMV than is Col-0. The data with CM4-184 are consistent with what has been observed previously (30,33). As with Col-0, viral DNA from CaMV isolate D4 was not detected in any part of En-2 plants.

The plant skeleton hybridization data for symptomatic En-2 plants generally reflected the symptom data (Table 1). NY8153 viral DNA was found throughout En-2 plants (Fig. 2). NY8153 DNA was also found in the cauline leaves and flower stalks of certain asymptomatic En-2 plants. These data indicate that NY8153 spreads through En-2 plants more efficiently than the other CaMV isolates, but some variability exists in the type of symptoms induced and the distribution of virus.

One CaMV isolate was able to produce a limited systemic infection in En-2 plants, even though it failed to induce symptoms.

Fig. 3. Cauliflower mosaic caulimovirus isolate CM1841 can spread through En-2 plants to a limited extent. **A**, Col-0 and **B**, En-2 plants inoculated with CM1841 were harvested at 52 days postinoculation and analyzed by the plant skeleton hybridization technique. **C**, A mock-inoculated En-2 control plant. Arrows indicate inoculated leaves; arrowheads, flower buds. Note, the plant in **C** was overexposed to detect the image.

CM1841 viral DNA was detected in the inoculated leaves, flower stalks, and flower buds of En-2 plants (Fig. 3). These data indicate that CM1841 can propagate in the inoculated leaves of En-2 plants and spread through the vascular system, but that the latter process is inefficient. Preliminary data with the Bari-1 isolate of CaMV (25) indicate that it can also spread, to a limited extent, through En-2 plants (data not shown).

Specificity of resistance. *A. thaliana* ecotype En-2 was resistant to most of the isolates of CaMV that were tested. The resistance exhibited by this ecotype could be due either to a specific response to CaMV or to reduced susceptibility to viruses in general. To test these possibilities, we inoculated Col-0 and En-2 plants with TuMV (54). *A. thaliana* Col-0 plants were inoculated as susceptible controls. TuMV induced severe stunting and chlorosis of the rosette leaves on both ecotypes by 18 DPI. By 22 DPI, the symptoms induced by TuMV were even more prominent (Fig. 4). All TuMV-inoculated plants died by 30 DPI without flowering. Thus, ecotypes of *A. thaliana* that varied in their resistance to CaMV were equally susceptible to TuMV. These data indicate that the resistance shown by *A. thaliana* ecotype En-2 was specific to CaMV. In support of this conclusion, preliminary data indicate that the En-2 and Col-0 ecotypes are also susceptible to turnip crinkle carmovirus (TCV) (data not shown).

DISCUSSION

In this study, we found that the resistant *A. thaliana* ecotype, En-2, responded differently to nine CaMV isolates. Specific CaMV

isolates such as NY8153 overcame resistance and induced visible symptoms in En-2. Other viral isolates appeared to spread to a limited extent through resistant plants, but did not induce symptoms, e.g., CM1841 in En-2. Still other isolates appeared to be limited to the inoculated leaves and were not capable of spreading through the resistant plants. Finally, the D4 CaMV isolate was not able to infect even the susceptible Col-0 ecotype.

Our results indicate that virus resistance in the En-2 ecotype is not an all-or-none response. Rather, there are different degrees of virus resistance within this ecotype, depending on the viral isolate inoculated to it. These results are perhaps not surprising, since it has been shown in other systems that plants preventing virus long-distance movement do permit limited spread of viruses (14,19,28).

It was previously shown that the long-distance movement of CM4-184 was prevented in En-2 plants (33), but it was not known if these plants use the same mechanism to stop all CaMV isolates. Our data indicate that En-2 plants do indeed utilize the same mechanism to produce resistance to most of the CaMV isolates tested. This suggests that resistance in *A. thaliana* is commonly mediated by inhibiting CaMV long-distance movement. The resistant Sv-0 and Tsu-0 ecotypes (30) also appear to prevent many CaMV isolates from producing a systemic infection, probably by inhibiting virus long-distance movement (data not shown). Prevention of CaMV long-distance movement in other hosts has also been reported (38).

Although the *CAR1* resistance gene in En-2 plants prevented most CaMV isolates from producing a systemic infection, NY8153 was able to completely break En-2 resistance. This virus was able to spread through En-2 plants, producing a severe systemic infection and invading every organ. These data indicate that CaMV isolate NY8153 is more "competent" in long-distance movement through En-2 plants than the other CaMV isolates examined in this study.

CaMV isolates are normally unable to produce a systemic infection in En-2 plants, because virus movement is prevented or because these resistant plants mount a defense response that limits the virus (5). En-2 plants could prevent CaMV movement by either lacking functions required by the virus to spread or by expressing factors that interfere with the movement of the virus (5). If En-2 resistance is mediated in this way, then NY8153 may be able to use other functions to move or its spread may not be interfered with by host factors in En-2 plants. This would result in efficient movement of the NY8153 virus through the plasmodesmata of the long-distance movement pathway in En-2 plants and in the production of a severe systemic infection.

CaMV infection has been shown to induce the expression of defense genes in En-2 plants (5). The induction of defense genes in susceptible plants delayed the rate at which CaMV produced a systemic infection, so this may explain En-2 resistance. In this scenario, the NY8153 virus may not activate the *CAR1* gene-mediated resistance pathway. Other virus strains have been proposed to overcome resistance by this mechanism (10,27). It is also possible that NY8153 gene products may inhibit the defense response, as has been shown for certain animal viruses (18). In either case, En-2 plants would have a reduced ability to mount a resistance response against NY8153, and an efficient systemic viral infection would result.

Interestingly, certain En-2 plants inoculated with NY8153 contained viral DNA in the flower stalk and cauline leaves, but did not produce symptoms. Because all of the En-2 plants for an experiment were at the same developmental stage at the time of inoculation and they developed at the same rate, it is possible that the NY8153 inoculum may have contained two or more virus strains. One of these putative strains is somewhat restricted in its distribution, whereas the other can spread throughout En-2 plants. Both of these hypothetical strains, however, spread more efficiently than the other CaMV isolates tested here.

Certain viral genes must control the long-distance movement of NY8153 in resistant En-2 plants. Two possible viral genes condi-

Fig. 4. En-2 resistance is specific to cauliflower mosaic caulimovirus. **A and B,** Col-0 and **C and D,** En-2 plants either **B and D,** mock-inoculated or **A and C,** inoculated with turnip mosaic potyvirus and photographed at 22 days postinoculation.

tioning the ability of NY8153 to move long distance in En-2 plants are gene II (discussed below) and gene VI. Gene VI is important in controlling CaMV host range, and it has been implicated in conditioning virus long-distance movement (45,49,50). However, preliminary data with viral chimeras (provided by J. Schoelz) indicate that NY8153 gene VI is not sufficient for breaking En-2 resistance. The role of the other CaMV genes in long-distance movement in resistant En-2 plants is unknown.

Some CaMV isolates (e.g., CM1841) were able to spread through En-2 plants to a limited extent. In these cases, the viral DNA was detected in the flower stalk and sometimes in the flower buds. Since no symptoms were observed, but viral DNA was detected in the flower stalk, it is likely that the levels of virus present in these organs were too low to induce visible symptoms. The distribution of virus in these situations is consistent with En-2 impairing an aspect of CaMV long-distance movement. If virus entry into the vascular system is delayed for a long enough time, only a small portion of the plant will be invaded by the virus, due to developmental resistance (33). Since the flower stalks and flower buds are the strongest sinks within the plant (22) and viruses move from source to sink (31,32), it makes sense for virus to be present in these organs. Hence, long-distance movement of the CM1841 CaMV isolate seems to be extremely delayed in En-2 plants.

CM1841 has been reported to invade the cauline leaves in En-2 plants (5), but virus was not detected in these organs in the study reported here. This difference could have at least two possible explanations. First, CM1841 was detected in the cauline leaves of infected En-2 plants using PCR, and it is possible that this technique is more sensitive than the plant skeleton hybridization technique used in this study. Second, in the work of Callaway et al. (5), En-2 plants were infected with CM1841 by agroinoculation, a more efficient type of inoculation than the mechanical method employed in this paper. It is known that certain geminiviruses that are difficult to inoculate to plants mechanically can produce a systemic infection if they are agroinoculated (20). Perhaps a similar phenomenon occurs with agroinoculation of CM1841 to En-2 plants. However, the reasons for increased infection efficiency of viruses delivered by agroinoculation, as compared with mechanical inoculation of virions, are not clear.

The results with CM1841 are also interesting, because they may suggest a potential role for CaMV gene II in long-distance movement. CaMV isolate CM1841 induced a limited systemic infection in En-2 plants, whereas its close relative CM4-184 was confined to the inoculated leaf (30,33). The genomes of CM4-184 and CM1841 are very similar; in fact, CM4-184 has been thought to be derived from CM1841 (23). Since CM4-184 contains a 421-base pair deletion in gene II (23), but CM1841 does not (16), it is tempting to speculate that CaMV gene II may play a role in systemic infection of En-2 plants. Gene II is the helper component for aphid transmission of CaMV (3,26,36,47), and it has been shown that the helper component proteinase gene of tobacco etch potyvirus functions in long-distance virus movement (9). In addition, both the gene II product (4) and the TMV movement protein have been shown to interact with microtubules (21). This may be further evidence that the CaMV gene II product plays a role in virus movement. Finally, gene II influences the host specificity of CaMV in a light-dependent manner, and these effects may be mediated by affecting long-distance movement (46). It is known that CM1841 produces small amounts of gene II product, and the virus is nonaphid transmissible for this reason (3), so it is possible that expression to higher levels may allow the virus to spread more efficiently and break resistance of En-2 plants. In support of this, NY8153 is aphid-transmissible (35), and it is able to break resistance in En-2 plants. Other aphid-transmissible CaMV isolates such as Cabb B (35), however, were not able to spread long distance through En-2 plants, even to a limited extent. Therefore, the role of CaMV gene II in long-distance movement in En-2 plants remains to be determined.

En-2 plants are less susceptible to CaMV isolates than other ecotypes such as Col-0 (5). However, the resistance shown by En-2 to CaMV is not due to an overall decrease in general susceptibility to viruses in this ecotype. En-2 plants were just as susceptible to an unrelated virus, TuMV, as those of the Col-0 ecotype. This suggests that the plant factors preventing systemic infection may be unique for different types of viruses. A similar phenomenon was observed in *A. thaliana* with TCV; the Di-0 ecotype was resistant to TCV, but not to other viruses (43).

The discovery of viral isolates that can overcome specific resistance genes now affords the opportunity to identify the viral determinants recognized by the host. It has been possible to identify CaMV genes responsible for controlling specific parts of the infection process by producing chimeras (45,50,51). Indeed, CaMV gene VI was identified as the viral gene responsible for overcoming resistance in solanaceous plants by this method. Therefore, it should be possible to use this same approach to identify the viral genes involved in breaking En-2 resistance.

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