An interspecies hybrid RNA virus is significantly more virulent than either parental virus

(plant RNA viruses/cucumber mosaic virus/hybrid virus/virus movement/virus synergism)

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ABSTRACT Cucumber mosaic cucumovirus (CMV) infects a very wide range of plant species (>1000 species). We recently demonstrated that a previously undescribed gene (2b) encoded by RNA 2 of the tripartite RNA genome of CMV is required for systemic virus spread and disease induction in its hosts. Herein we report that when this CMV gene is replaced by its homologue from tomato aspermy cucumovirus (TAV), the resultant hybrid virus is significantly more virulent, induces earlier onset of systemic symptoms, and accumulates to a higher level in seven host species from three families than either of the parents. Our results indicate that CMV and the TAV 2b protein interact synergistically despite the fact that no synergism occurs in double infections with the two parental viruses. To our knowledge, this is the first example of an interspecific hybrid made from plant or animal RNA viruses that is more efficient in systemic infection of a number of hosts than the parental viruses. The relevance of our finding to the application of pathogen-derived resistance is discussed.

Virulence is undoubtedly an agroeconomically important property of plant viruses. However, the mechanisms by which viruses induce disease symptoms are poorly understood. Efficient infection by in vitro-transcribed RNAs (1) or directly by plasmid DNAs (2) of full-length cDNA clones of plant RNA viruses has made it possible to manipulate RNA genomes and to investigate the molecular basis of pathogenicity and virulence. In recent years, mutations introduced into various regions of the viral genome have been found to influence symptom expression. These include mutations in regions of the viral genome coding for the viral replicate (3–6), movement protein (7–9), and coat protein (10–12), as well as in the 5′ (13) or 3′ (14) untranslated regions.

We have recently discovered (15) a small overlapping gene (2b) encoded by RNA 2 of the tripartite RNA genome of all cucumoviruses sequenced to date that is absent in closely related viruses such as the bromoviruses. The open reading frame 2b (ORF 2b; Fig. 1a) located at the 3′ end of RNA 2 overlaps, but is out of frame with, the C-terminal region of ORF 2a encoding the viral RNA polymerase protein. The in vitro expression of the 2b gene, most likely through the subgenomic mRNA RNA 4A (Fig. 1a), has been shown for both CMV and TAV cucumoviruses (unpublished data; ref. 15).

A recent mutational analysis has shown that the 2b gene of CMV facilitates long-distance movement of the virus throughout infected plants and that 2b is an important virulence determinant in cucumber and Nicotiana glutinosa (16). We herein report that replacing the 2b gene of CMV with its homologue from TAV transforms a poorly virulent CMV strain into a hybrid virus that is very virulent in all seven plant species tested. To our knowledge, this is the first example of an interspecies hybrid made from plant or animal RNA viruses that is more efficient in systemic infection of a number of hosts than the parental viruses.

MATERIALS AND METHODS

Plasmid Constructs. Plasmids pQCD1, pQCD2, and pQCD3 used for efficiently producing CMV infection have been described (2). Plasmid pQCD2qt, a derivative of pQCD2, was obtained using three overlapping PCR fragments I, II, and III (boxed with dotted line, Fig. 1b) essentially as described (17). Fragment II, representing the coding sequence of TAV ORF 2b (nt 2447–2734 of TAV RNA 2; ref. 18), was obtained from the virion RNAs by reverse transcription-coupled PCR as described (16) using primers SD22 and SD23 (Table 1). Fragments I and III flank CMV ORF 2b (nt 2410–2712) and represent nt 2050–2411 and nt 2713–3035 of CMV RNA 2, respectively; these two fragments were obtained by two separate PCRs using pQCD2 as the template and primer pairs SD45/SD24 and SD25/SD6, respectively. SD6 and SD24 are complementary to nt 3011–3035 and nt 2388–2409, whereas SD25 and SD45 correspond to nt 2711–2730 and nt 2050–2075 of CMV RNA 2 (2).

Fragments I, II, and III were assembled into the hybrid fragment approximately 970 bp long in a final PCR using primers SD45/SD6, as fragment II contained sequences at its termini (shown as thinner extensions in Fig. 1b) that overlap I and III. This overlap was defined by the chimeric primers SD22 and SD23 (Table 1) used to generate fragment II. This PCR product was digested by Asp718 (Boeringer Mannheim) and the resultant Asp718 fragment was used to substitute the wild-type Asp718 fragment (corresponds to nt 2094–2904 of CMV RNA 2) of pQCD2 to yield pQCD2qt.

Specific mutations were introduced into pQCD2qt to create pQCD2qt1 and pQCD2qt2 by a PCR mutagenesis protocol as described (16). The sequences of mutagenic primers used are listed in Table 1. The Asp718 fragments in all three constructs (pQCD2qt, pQCD2qt1, and pQCD2qt2) that had been manipulated by PCR were completely sequenced before use.

Plant Inoculation and Virus Progeny Analysis. We have described (2, 16) methods for inoculating N. glutinosa and Nicotiana tabacum cv. Samsun plants with infectious plasmid DNAs, Northern blot hybridization, and virus progeny RNA analysis by direct RNA dideoxynucleotide sequencing and by sequencing the cloned cDNA obtained by reverse transcription-coupled PCR. Virions (0.2 mg/ml) of CMV (Q strain; ref.

Abbreviations: CMV, cucumber mosaic cucumovirus; TAV, tomato aspermy cucumovirus; ORF, open reading frame.
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Table 1. Mutagenic primers used for generating mutant constructs of RNA 2

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<tr>
<th>Primer</th>
<th>Primer Sequence</th>
<th>Construct</th>
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<tr>
<td>SD22</td>
<td>5'-ACCGTTAGAAGAAGAAGAATGGCCAGCTCGGATCCTCC-3' (CMV RNA 2 nt 2391-2409)</td>
<td>pQCD2qt</td>
</tr>
<tr>
<td>SD23</td>
<td>5'-CGGAGCAGAAGGTTGCAGAAATCAGGGACTCACTGACG-3' (CMV RNA 2 nt 2713-2733)</td>
<td>pQCD2qt</td>
</tr>
<tr>
<td>SD43</td>
<td>5'-ACCGTATAAGGAAGTGAGAAGT-3' (CMV-qt RNA 2 nt 2391-2411)</td>
<td>pQCD2qt1</td>
</tr>
<tr>
<td>SD47</td>
<td>5'-TAAGAAGTTAGAAGTGAGAAGT-3' (CMV-qt RNA 2 nt 2396-2425)</td>
<td>pQCD2qt2</td>
</tr>
</tbody>
</table>

Underlined nucleotides in the SD43 and SD47 sequences specify the mutations introduced into each of the mutant constructs. The SD23 primer sequence is complementary to the specified regions of CMV or TAV; all others are plus sense. Primers SD44 and SD48 (not given) are the exact complement of SD43 and SD47, respectively.

2), TAV (V strain; ref. 18), or the hybrid viruses were purified from the N. glutinosa plants infected by the plasmids and used to inoculate seedlings of Datura stramonium, Gomphrena globosa, Lycopersicon esculentum, Physalis floridana, and Seltaria media.

Preparation and Transfection of Tobacco Protoplasts. Exponentially growing tobacco NT-1 suspension cells (N. tabacum) were used to prepare protoplasts as described (19). From 300,000 to 400,000 protoplasts were transfected using a PEG method (20) by 4 μg of RNA isolated from purified virions. The protoplasts were collected immediately after transfection and washing or after incubation in the protoplast incubation medium (19) for 24 h at 26°C in dark, and protoplast RNAs were extracted using TRIzol reagent (GIBCO/BRL) and examined by Northern blot analysis.

RESULTS

We have shown (16) that the 2b gene encoded by CMV (Fig. 1a) is required for long-distance virus movement in cucumber and for causing disease in N. glutinosa. To investigate whether these functions encoded by the 2b gene of CMV can be replaced by the homologue from a different cucumovirus, a hybrid RNA 2 cDNA clone, pQCD2qt, was constructed. The structure of pQCD2qt is shown in Fig. 1b and was the same as that of pQCD2 (the wild-type CMV RNA 2 cDNA clone) except for the ORF 2b coding sequence, which was from TAV. The amino acid sequences encoded by ORF 2b of TAV and CMV are 22.6% identical, the most different pair of known cucumoviruses (15).

Characterization of the Hybrid Virus in the Infected Tobacco (N. tabacum) Plants. pQCD2qt, like pQCD2, was bio logically active when coinoculated with pQCD1 and pQCD3 (the wild-type cDNA clones of CMV RNAs 1 and 3) as the resultant hybrid virus (CMV-qt) accumulated in systemically infected leaves (upper uninoculated leaves) of the inoculated tobacco plants (Fig. 2a, lane 3). To examine the nature of the progeny RNA 2, total RNAs were extracted from systemic leaves 12 days after inoculation and parallel Northern blot hybridization reactions were done using cRNA probes specific for the 3' ends of all CMV positive-strand RNAs (Fig. 2a) and for the positive strands of the TAV 2b gene (Fig. 2d). Three genomic RNAs and two subgenomic RNAs known to be associated with CMV infection (Fig. 2a, lane 2; refs. 2 and 16) were detected in the tobacco plants infected with CMV-qt (Fig. 2a, lane 3). Of these viral RNAs, RNAs 2 and 4A also hybridized to the TAV 2b gene-specific probe (Fig. 2d, lane 16), indicating the presence of the engineered TAV 2b gene in the progeny. The specificity of this TAV 2b gene probe was demonstrated by the absence of hybridization signal with any of CMV RNAs (Fig. 2d, lane 15) and by its hybridization only with RNAs 2 and 4A of the five major TAV RNAs (Fig. 2d, lane 17).

The 3'-terminal sequence of 667 nt (including the inserted TAV sequence) of the progeny RNA 2 derived from pQCD2qt (Fig. 1b) was completely determined from three cDNA clones obtained by reverse transcription-coupled PCR. The data shown that the progeny RNA 2 contained the complete ORF 2b coding sequence from TAV, but not the CMV ORF 2b coding sequence, and that the nucleotide sequence of this region of the progeny RNA 2 was identical to the corresponding region of pQCD2qt. This result not only further confirmed the chimeric nature of the progeny RNA 2 but also indicated that the engineered TAV sequence had been stably and accurately maintained as an integrated part of the hybrid viral RNA genome.

![Fig. 1. Genome organization of species of the cucumovirus group and structure of cucumber mosaic cucumovirus (CMV) (Q strain) RNA 2 wild-type or mutant cDNA clones used for virus infection. (a) Genome organization of cucumoviruses. Proteins 1a, 2a, and 3a are translated in vivo from genomic RNAs 1, 2, and 3, respectively. The coat protein (CP) and protein 2b are translated from the subgenomic mRNAs, RNAs 4 and 4A, respectively. (b) The structure of plasmids pQCD2 and its derivatives. Plasmids pQCD2qt (or its derivatives), as well as plasmids pQCD1 and pQCD3, contain the full-length DNA copies of RNAs 2, 1, and 3, respectively, under the transcriptional control of the 35S promoter (P) and terminator (T) of cauliflower mosaic virus. The insert boxed with dotted lines shows the three PCR fragments (enlarged for clarity) used for constructing pQCD2qt. The numbers with an arrow above or underneath represent the names of primers in the SD series. The thin lines represent CMV RNA sequence, whereas the thick central black bar depicts the tomato aspersy monocucumovirus (TAV) RNA sequence encoding the 2b protein. The blank area surrounded by dashed lines in ORF 2a (pQCD2qt1 and pQCD2qt2) or ORF 2b (pQCD2qt2) represents deletions in the ORF only by single nucleotide substitution(s).]
The chimeric RNAs 2 and 4A were also detected by Northern blot analysis in virions purified from plants infected with CMV-qt. A primer (5'-TCTCGTGTAGGGGTCT-3') that binds to a region of the chimeric RNA 2 that is 12 nt to the 3' side of the start codon of the TAV ORF 2b was used in a primer-extension experiment that showed that the 5' initiation site of the chimeric RNA 4A was identical to that of CMV RNA 4A (data not shown). Thus, replacing the CMV ORF 2b coding sequence with the homologous TAV sequence had no obvious effect on either the encapsidation or the transcriptional initiation of RNA 4A.

An antisera, raised essentially as described (15) against the TAV 2b protein that was fused with glutathione S-transferase and produced in Escherichia coli, detected the 2b protein of 14 kDa in the TAV-infected plants by Western blot analysis (unpublished data). A protein species of the same mobility as the TAV 2b protein was also detected by this antisera in protein extracts of the tobacco plants infected by CMV-qt; this unequivocally demonstrated the in vivo expression of TAV ORF 2b from CMV-qt.

The Hybrid Virus Is Highly Virulent in Tobacco Plants. In the glasshouse conditions we used, systemic infection of tobacco plants (N. tabacum cv. Samsun NN) with CMV produced only light molting that was hardly visible and the infected plants appeared healthy (Fig. 3a). However, tobacco plants infected with CMV-qt showed severe symptoms (Fig. 3a); leaves were distorted and the plants were dwarfed. At later stages of infection, the infected plants produced fern-leaf symptoms. CMV-qt was also significantly more virulent than TAV, although TAV induced clearly visible symptoms as it also did in P. floridana (Fig. 3b; see below). Importantly, CMV-qt induced systemic symptoms to appear rapidly, at least 3 days earlier than those induced by TAV. Further, the genomic RNAs of CMV-qt clearly accumulated to a much greater concentration in infected tobacco plants than did those of CMV (Fig. 2a, compare lanes 2 and 3). This increase in viral RNA accumulation correlates with the hypervirulence of CMV-qt.

The molecular basis of increased viral RNA accumulation in infected plants was followed in tobacco protoplasts inoculated with RNAs prepared from purified viral particles of CMV and CMV-qt. Infectious plasmid DNAs were not used because viral RNA transcripts must first be transcribed from these plasmids, presumably in the nuclei, and transported back to the cytoplasm before a normal cycle of viral gene expression/replication can begin; these additional processes may have an effect on the profile of virus replication if monitored for only a short period after inoculation. Northern blot analysis of total RNAs extracted from the protoplasts immediately after inoculation detected only trace amounts of viral genomic RNAs. However, after a 24-h incubation at 26°C, a significant amount of viral genomic and subgenomic RNAs accumulated in the protoplasts that had been inoculated with CMV virion RNAs (Fig. 2e, lane 11), showing that the introduced viral RNAs replicated in these protoplasts. Clearer CMV-qt (lane 12) and CMV (lane 11) accumulated RNA to a similar level, thus, indicating that the greater amount of CMV-qt RNAs compared with CMV RNAs in infected plants was unlikely to result from differences in the relative rates of virus replication.

The Unusual Properties of the Hybrid Virus Result from a Synergistic Interaction Between CMV and the TAV 2b Protein. As ORF 2b overlaps ORF 2a, the TAV RNA sequence of the hybrid RNA 2 derived from pQCD2qt was expected to be translated in two reading frames and give the TAV 2b protein, as well as the C-terminal 41 amino acids of the 92-kDa chimeric 2a protein (Fig. 1b). Therefore, two mutants of pQCD2qt were constructed to determine whether the unusual properties of CMV-qt (hypervirulence, increased accumulation, and rapid appearance of systemic symptoms) were caused by (i) the TAV 2b protein, (ii) the chimeric 2a protein, or (iii) the TAV RNA sequence. A stop codon was introduced in ORF 2a immediately to the 5' side of the TAV sequence of pQCD2qt to yield pQCD2qt1 (Fig. 1b). The second codon (GCA) of ORF 2b in pQCD2qt1 was further changed to a stop codon (UAU) to give pQCD2qt2 (Fig. 1b).

CMV-qt1 (derived from inoculum pQCD2qt1 + pQCD1 + pQCD3) also induced the rapid appearance of severe systemic symptoms in tobacco (Fig. 3a) and was thus phenotypically similar to CMV-qt. In contrast, CMV-qt2 (derived from inoculum pQCD2qt2 + pQCD1 + pQCD3) was similar in virulence to CMV and caused poorly visible symptoms in tobacco. Further, CMV-qt1, like CMV-qt, accumulated to a much greater concentration than CMV did in infected tobacco (Fig. 2a, compare lanes 3 and 4 with lane 2) in spite of a similar level of accumulation of all viruses in infected protoplasts (Fig. 2c). Therefore, the unusual properties of CMV-qt correlated directly with the expression of the TAV 2b protein that was regulated by the cis-acting elements of CMV. These results indicate that it was the synergistic interaction of the TAV 2b protein, not the inserted RNA or the chimeric 2a protein, with CMV (devoid of its own 2b) that led to the hypervirulence of CMV-qt. However, plants that were doubly infected with the

![Fig. 2. Accumulation of the RNAs of CMV, TAV, and their hybrid viruses in infected plants and tobacco protoplasts. Five micrograms of RNA, isolated from systemic leaves of N. tabacum (a and d) or P. floridana (b) plants 12 days after inoculation, was loaded in each lane except for lanes 3 and 4, in which only 1 µg of RNA was loaded. Each lane in c contained RNA extracted from about 200,000 protoplasts after inoculation and 24 h of incubation. The letter(s) above each lane indicate(s) the source of inoculum. H or 0, uninoculated controls; C, CMV; qt, CMV-qt; qt1, CMV-qt; qt2, CMV-qt2; T, TAV; 2a', CMV-2a'. a–c were probed by the CMV 3'-end cRNA probe, which is complementary to the 3'-terminal 340 nt of CMV RNA 2, for assessing accumulation of all viral RNAs of CMV or its derivatives; this probe also hybridized to TAV RNAs (lane 6). d was probed with a cRNA probe complementary to nt 2447–2651 of TAV RNA 2 (ref. 18) to determine accumulation of viral genomic and subgenomic RNAs containing the TAV ORF 2b coding sequence: RNAs 2 and 4A of CMV-qt (lane 16) and TAV (lane 17). The positions of viral RNAs 1, 2, 3, 4, and 4A are indicated between c and d.](image-url)
parents of CMV-qt (CMV and TAV), as demonstrated by Northern blot analysis, did not show the synergistic effect.

Protoplast infection experiments also indicated that the C-terminal overlapping portion of the 2a protein (Fig. 1b) was not required for viral replication because a similar amount of viral RNA accumulated in protoplasts (Fig. 2c) infected with CMV (lane 11), CMV-qt (lane 12), CMV-qt1 (lane 13), or CMV-2a' (lane 14). CMV-2a', a mutant of CMV, contained a point mutation that resulted in an early termination of ORF 2a immediately before the start of ORF 2b (16).

The Hybrid Virus Is Highly Virulent to Six Additional Plant Species from Three Families. The susceptibility of six additional host species belonging to three families (Table 2) was tested to determine whether the synergistic responses associated with CMV-qt infection were specific to particular host species. CMV did not cause obvious symptoms to any of the hosts listed in Table 2 except N. glutinosa although it infected them all. TAV induced clearly visible symptoms in P. floridana, L. esculentum, and N. glutinosa (Fig. 3b for P. floridana; data not shown) in addition to N. tabacum as described above. The results obtained, as summarized in Table 2, showed that CMV-qt induced rapid appearance of severe systemic symptoms (Fig. 3b for P. floridana) and the viral RNAs accumulated to high levels (Fig. 2b, lane 8 for P. floridana) in all of these plants. Thus, these six host species responded to CMV-qt infection in the same way as the tobacco plants as described above. In the host species that displayed visible symptoms when infected with CMV or TAV (Table 2), the systemic symptoms induced by CMV-qt were significantly more severe (Fig. 3b) and appeared at least 3 days earlier than those caused by infection with the parental viruses. The observed features of the CMV-qt infection in N. glutinosa also indicate that the TAV 2b gene is functionally similar to the 2b gene of CMV since a CMV mutant expressing no 2b protein induced a delayed appearance of the very mild systemic symptoms in this host compared with wild-type CMV (16).

Further infection experiments were done with these six hosts using the mutants CMV-qt, CMV-qt1, and CMV-qt2. The results obtained agreed with those from infection experiments in tobacco plants described above (Figs. 2a and 3a) in that the hypervirulence and enhanced accumulation of CMV-qt RNAs in infected plants correlated with the expression of TAV 2b protein because CMV-qt1 (Fig. 2b for P. floridana), but not CMV-qt2, accumulated to high levels and induced rapid appearance of severe systemic symptoms (Fig. 3b for P. floridana) in all of the six hosts. This is also consistent with our finding (16) that the 2b protein of CMV is essential but that the overlapping C-terminal portion of protein 2a is dispensable for symptom expression of CMV in N. glutinosa and cucumber.

DISCUSSION

The genome structure of the hybrid virus (CMV-qt) described in this report is identical to that of CMV except for the small overlapping gene 2b. This gene was transferred from TAV and constitutes only 3.3% of the complete CMV-qt genome. We have shown that CMV-qt was significantly more virulent and induced earlier onset of systemic symptoms than either of the parental viruses in seven plant species belonging to three families (Table 2). CMV-qt RNAs clearly accumulated to a higher level in the infected plants than did those of CMV (Fig. 2). To our knowledge, this is the first example of an interspecies hybrid made from plant (21–24) or animal (25–27) RNA viruses that is more efficient in systemic infection of a number of hosts than the naturally selected parents. We are currently determining whether CMV-qt is also more competitive than its parental viruses and becomes the dominant isolate when plants are doubly infected with CMV-qt and CMV or TAV.

Our results indicate that the hypervirulence of CMV-qt most likely resulted from a synergistic interaction between the TAV 2b protein and CMV (or precisely, CMV in the absence of its own 2b). It is known that certain plant viruses can interact with each other synergistically leading to enhanced virulence (28). However, as double infection with CMV and TAV induced no synergistic response, the interaction we observed represents a new type of virus synergism; namely, one plant virus can interact synergistically with a protein derived from a second virus and encoded in cis even though no virus synergy occurs in double infections.

Table 2. Infectivity of CMV, TAV, and the hybrid virus in seven host species belonging to three families

<table>
<thead>
<tr>
<th>Virus</th>
<th>Nicotiana tabacum*</th>
<th>Nicotiana glutinosa*</th>
<th>Datura stramonium*</th>
<th>Lycopersicon esculentum*</th>
<th>Physalis floridana*</th>
<th>Stellaria media†</th>
<th>Gomphrena globosa‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>CMV</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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Three levels of virulence were scored: +, systemic infection with essentially no symptoms; ++, clearly visible systemic symptoms; ++++, severe systemic symptoms. The host plants listed here belong to the following three families, *Solanaceae; †Caryophyllaceae; ‡Amaranthaceae.
What is the molecular basis of the observed synergism? Infection experiments in tobacco protoplasts showed that substituting the 2b gene in RNA 2 of CMV with that of TAV had no detectable effect on accumulation of the genomic RNAs 1 and 3 of CMV, indicating that the synergism was unrelated to changes in viral replication. The TAV 2b protein may act as a cytopathic agent within the plant, at least in the host species examined, and as a result, the hybrid virus spreads much more quickly in infected plants. This is partly supported by differences in the timing when _N. glutinosa_ plants first showed systemic symptoms after inoculation with CMV-qt or CMV since CMV-qt induced the appearance of systemic symptoms at least 3 days earlier than did CMV. The 2b gene of CMV has been shown to encode a long-distance virus movement function (16); thus, it is highly likely that its homologue encoded by TAV has a similar function. As all seven species examined are also hosts of TAV (Table 2), the 2b gene from TAV should have been well adapted to function in these hosts. Moreover, it is considered that symptom severity is largely determined by the relative rate of virus movement and the rate of plant growth (29); further, efficient virus movement will lead to an increased number of cells being infected and thus to increased viral accumulation. It has been shown that single amino acid changes in the movement proteins of other plant RNA viruses can dramatically alter both viral accumulation and virulence (8, 30). Therefore, the synergistic responses associated with CMV-qt infection, including hypervirulence, enhanced virus accumulation, and rapid appearance of systemic symptoms, can all result from an increased speed of CMV-qt systemic spread through infected plants compared with CMV.

CMV-qt was also much more virulent than TAV, one of the parent viruses of CMV-qt. This is most likely to result from a greater amount of the TAV 2b protein being expressed by the CMV-qt genome than by the TAV genome as was shown by Western blot analysis (unpublished data). As shown in Fig. 2d, a greater amount of the chimeric RNA 4A (mRNA of 2b) accumulated in plants infected with CMV-qt (lane 16) than that of RNA 4A in the TAV-infected plants (lane 17). This may reflect the relative strength of the CMV and TAV subgenomic RNA promoters (15) controlling transcription of RNA 4A from RNA 2. This difference in the amount of the TAV 2b protein produced by TAV and by CMV-qt may also explain why no synergistic interaction occurred in double infection with CMV and TAV where expression of the TAV 2b protein was controlled by TAV genome.

Several control strategies utilize transgenic expression of viral proteins (e.g., coat protein, replicase, and movement proteins) to protect transgenic plants from infection by the source virus (31). Our finding raises an important point in utilizing these types of transgenic resistance since functional viral proteins expressing from transgenic plants may interact synergistically with heterologous invading viruses in the field. This type of virus synergism would make the transgenic plants more severely affected than the nontransgenic plants. Two reports further indicate that this synergism can occur in transgenic plants; it has been most recently shown that part of the potyviral genome expressed from transgenic plants is sufficient to induce the known potato virus X/potyviral synergistic disease (32) and that the disease development of CMV was accelerated in transgenic tobacco plants expressing the movement protein of tobacco mosaic virus (33). However, such potential interactions can be readily examined in risk assessment trials.

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