Symptom Intensification on Cruciferous Hosts by the Virulent Satellite RNA of Turnip Crinkle Virus

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ABSTRACT

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Satellite RNAs are small, single-stranded, linear, or circular RNAs that require a helper virus for replication. Turnip crinkle virus supports the replication of at least three satellite RNAs, one of which (sat-RNA C) was previously found to intensify viral symptoms on turnip cultivar Just Right. For this report, we have analyzed the ability of the virulent sat-RNA C to intensify symptoms on other cruciferous plants. Sat-RNA C was found to exacerbate symptoms on all hosts where TCV produced visible symptoms including cultivars of *Brassica rapa* and *Arabidopsis thaliana*. However, sat-RNA C did not have any intensifying effect on four cultivars of *B. rapa* that were tolerant to TCV infection. Lack of symptoms was not accompanied by lower accumulation of TCV or sat-RNA C. These results support our previous model that symptoms induced by TCV and sat-RNA C are the possible result of an analogous interaction between their similar 3′ end domains and an unidentified factor present in some hosts.

Turnip crinkle virus (TCV) is a single-stranded RNA virus of positive polarity recently classified as a member of the carmovirus group (14). Although TCV mainly infects members of the Crucifereae, it has a wide host range, including some 20 different plant families (3,9). TCV (4,051 bases) has recently been cDNA cloned. Sequence analysis revealed three open reading frames coding for four possible proteins, one being a readthrough product (4). Besides the 4-kb genomic RNA, natural strains of TCV can be associated with three small satellite RNAs (1,19) as well as defective-interfering (DI) RNAs (13). Sat-RNA D (194 bases) and sat-RNA F (231 bases) are avirulent satellites, whereas sat-RNA C (356 bases) is virulent and has been shown to intensify TCV symptoms on *Brassica rapa* spp. *rapifera* (turnip) 'Just Right' (1,18,20). Turnip plants infected with TCV, lacking sat-RNA C, exhibit symptoms consisting of mild stunting, slight leaf crinkling, and vein-clearing. The addition of sat-RNA C to the inoculum results in dark green plants that are severely stunted and have tightly crinkled leaves.

Sat-RNA C is an unusual hybrid molecule consisting of a 5′ domain very similar to full-length sat-RNAs D and F, and a 3′ domain composed of two regions at the 3′ end of TCV genomic RNA (19). Sat-RNAs D and F share no sequence similarity with TCV, with the exception of seven nucleotides located at the 3′ end of TCV and all three satellites. Studies with chimeric satellites containing the 5′ 155 bases of sat-RNA F ligated to the 3′ 200 bases of sat-RNA C revealed that the 3′ TCV-similar domain of sat-RNA C is responsible for symptom intensification by the satellite (17). This finding led to the hypothesis that the 3′ domain of sat-RNA C and, perhaps, the very similar 3′ untranslated region of TCV are responsible for symptom production by interacting or interfering with some unidentified component present in plant cells.

Many satellites have been identified that modulate the symptoms of their helper viruses (for recent reviews, see 10,16). Satellites associated with tobacco ringspot virus (8) and tomato bushy stunt virus (7) are implicated in the attenuation of disease symptoms produced by their helper viruses. Satellites associated with cucumber mosaic (11), arabis mosaic (5), and groundnut rosette viruses (15) intensify symptoms on specific hosts in an undetermined manner. To date, symptom intensification by sat-RNA C has only been demonstrated for turnip cultivar Just Right (1,20). We were interested in determining whether sat-RNA C modulated virus symptoms on other hosts of TCV. For this report, we have analyzed 23 cultivars from five species of crucifers for systemic viral spread as well as symptoms produced by TCV with or without sat-RNA C.

MATERIALS AND METHODS

Inocula. The wild-type strain of TCV containing sat-RNAs C, D, and F (TCV-M) was originally obtained from Roger Hull, John Innes Institute, England. TCV containing only sat-RNA D (TCV-m + D) was prepared by inoculating turnip with gel purified TCV. As previously reported (20), it has not been possible to maintain a stock of TCV without sat-RNA D.

Plant materials and growth conditions. Plants used in this study were obtained as seeds from the following sources: *B. rapa* spp. *chinensis* 'Pak Choi Joi Choi' (Park Seed), 'Hon Tsai Tai' (Sakata Seed), 'Sha Ho Tsai' (Sakata Seed), 'Tsai Shim' (Sakata Seed); *B. r. pekinensis* 'Michihili Jade Pagoda' (Park Seed), 'Green Rocket' (Park Seed); *B. r. perviridis* 'Tendergreen' (Park Seed), 'Savanna' (Park Seed); *B. r. rapifera* 'Just Right' (Burpee), 'Tokyo Cross' (Park Seed); *B. juncea* 'India' (Park Seed); *B. napus* 'American Purple' (Park Seed); *Arabidopsis thaliana* strains La-0, Col-0, Ag-0, Bus-0, No-0 (generous gift of F. Ausebel, Harvard Medical School); *Raphanus sativus* 'April Cross' (Park Seed), 'Inca' (Park Seed), 'Champion' (Park Seed), 'Cherry Belle' (Park Seed), 'French Breakfast' (Park Seed), and 'Icicle' (Park Seed).

Plants were grown in growth chambers under a 16-hr day. Temperature was 19 C day and 17 C night. The initial two leaves of 2-wk-old seedlings (with the exception of *A. thaliana*) were manually inoculated with 35 μl per leaf of buffer (50 mM glycine, pH 9.0, 30 mM K_2HPO_4, 0.02% bentonite, 0.5% Celite) or buffer including 5 μg of total plant RNA containing either TCV plus sat-RNAs C, D, and F (TCV-M) or TCV plus sat-RNA D (TCV-m + D). *A. thaliana* seedlings were inoculated at 20 days postgermination. Symptoms were generally first visible after 10 days. Scoring of symptoms was based on the analysis of at least six plants.

RNA extraction and analysis. RNA was extracted from leaves of approximately the same stage of maturity about 2 wk postinoculation as previously described (19), and subjected to electrophoresis on either 50% urea, 5% polyacrylamide gels in 0.5× TBE (1× is 45 mM Tris-borate, 45 mM boric acid, 1 mM EDTA) for satellite detection or 1% nondenaturing agarose gels in 0.5× TBE for genomic TCV detection. The concentration of total RNA in each sample was determined spectrophotometrically. Gels were stained with ethidium bromide for direct visualization. Photographic negatives were scanned with a soft laser scanning densitometer (Biomed Instruments Inc., Fullerton, CA) and levels of TCV and sat-RNA C normalized to internal rRNA controls in each lane.

RESULTS

Previous studies using turnip cultivar Just Right as a host indicated that sat-RNAs D and F were avirulent when inoculated

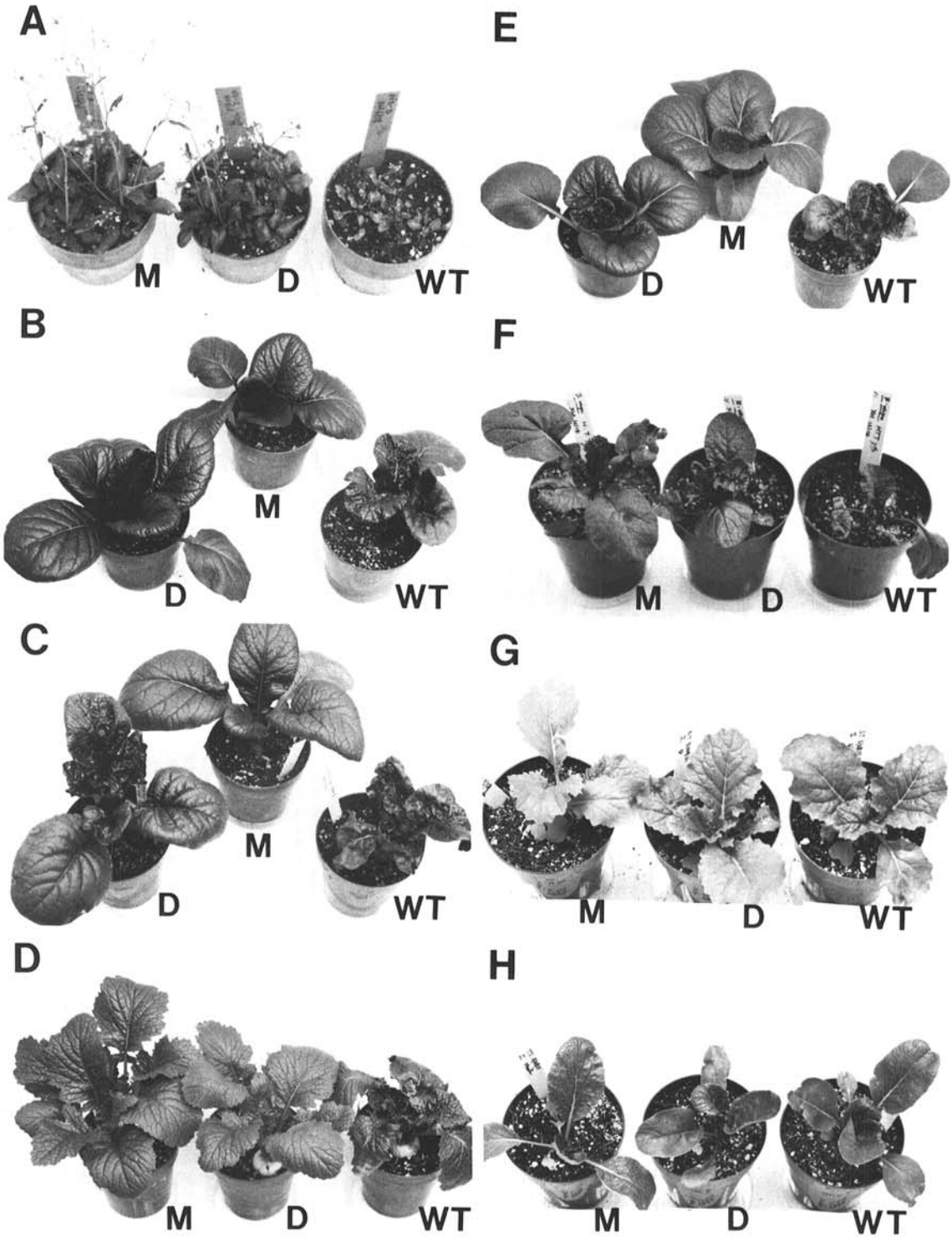

Fig. 1. Symptoms of selected crucifers infected with turnip crinkle virus plus either sat-RNAs C, D, and F (TCV-M) or sat-RNA D alone (TCV-m + D). Plants were photographed 2 wk after being inoculated with buffer (M), TCV-m + D (D) or TCV-M (WT). **A,** *Arabidopsis thaliana* No-0; **B,** *Brassica rapa* spp. *perviridis* 'Savanna'; **C,** *B. r. perviridis* 'Tendergreen'; **D,** *B. r. rapifera* 'Tokyo Cross'; **E,** *B. r. chinensis* 'Pak Choi Joi Choi'; **F,** *B. r. chinensis* 'Hon Tsai Tai'; **G,** *B. r. chinensis* 'Sha Ho Tsai'; **H,** *B. r. pekinensis* 'Tsai Shim'. There was no discernible difference in symptoms among different *A. thaliana* strains. Sha Ho Tsai and Tsai Shim cultivars were tolerant to TCV infection and exhibited no discernible symptoms whether or not sat-RNA C was included in the inoculum.

separately or together on seedlings along with gel-purified genomic TCV (1,20). Sat-RNA C, however, was virulent, intensifying the symptoms of TCV. In these experiments, it was not possible to maintain TCV free of sat-RNA D for more than a single passage, at most. When turnip was inoculated with virions isolated from plants infected with in vitro synthesized transcripts of TCV, sat-RNA D reappeared in approximately 50% of the infected plants (Li and Simon, *unpublished data*). Therefore, all TCV inoculums used in this study contained sat-RNA D to assure uniform results.

To determine the effect of sat-RNA C on various hosts other than turnip cultivar Just Right, potential TCV hosts were inoculated with buffer (mock) or buffer along with TCV preparations that included sat-RNA C, sat-RNA D, and sat-RNA F (TCV-M) or TCV that had been previously freed of sat-RNA C and sat-RNA F (TCV-m + D). Two to three weeks post-inoculation, plants were visually scored for the presence (or absence) of symptoms (Fig. 1, Table 1). On all hosts where symptoms were detected following inoculation with TCV-m + D, sat-RNA C was found to intensify these symptoms. Plants infected with TCV-m + D (with the exception of *A. thaliana*) exhibited mild symptoms consisting of slight stunting and leaf crinkling. Plants infected with TCV-M exhibited greater stunting and crinkling, as well as dark green leaves. Especially severe symptoms were associated with infection of all strains of *A. thaliana*. *A. thaliana* infected with TCV-m + D were more severely affected than other TCV-infected hosts, exhibiting symptoms including leaf browning, stunting, and inhibition of bolting. *A. thaliana* infected with TCV-M became increasingly necrotic and were dead by 18 days postinoculation (Fig. 1A). The results of these analyses are summarized in Table 1. As expected, not all crucifers were hosts for TCV. Although several cultivars of *R. sativus* (radish) were reported to be severely affected by infection of TCV (3) none of the cultivars of radish we inoculated exhibited symptoms, nor was the presence of TCV genomic RNA detected by acrylamide or agarose gel electrophoresis (data not shown).

All cultivars of *B. rapa* tested were hosts for TCV. However, not all cultivars exhibited symptoms in response to viral infection. Two of four cultivars of *B. r. chinensis* as well as both cultivars of *B. r. pekinensis* were tolerant to infection by TCV-m + D or TCV-M, exhibiting no visible symptoms over mock inoculated plants (Fig. 1G and H). To determine if the levels of viral and/or satellite accumulation were reduced in tolerant plants, total leaf RNA was prepared from plants 2 wk postinoculation and subjected to electrophoresis on 1% nondenaturing agarose gels for visualization of genomic TCV RNA or denaturing 5% polyacrylamide gels, for satellite detection (Fig. 2). Gels were stained

TABLE 1. Response of some cruciferous plants to inoculation with turnip crinkle virus (TCV) plus one or several of its satellite RNAs

Potential plant host	Presence of systemic viral infection	Expression or nonexpression of symptoms after inoculation with	
		TCV-m + D[a]	TCV-M[b]
Brassica rapa			
spp. *chinensis*			
Pak Choi hybrid	yes	+[c]	++
Hon Tsai Tai	yes	+	++
Sha Ho Tsai	yes	−	−
Tsai Shim	yes	−	−
spp. *pekinensis*			
Michihili Jade Pagoda	yes	−	−
Green Rocket hybrid	yes	−	−
spp. *perviridis*			
Tendergreen	yes	+	++
Savanna	yes	+	++
spp. *rapifera*			
Tokyo Cross	yes	+	++
Just Right	yes	+	++
B. juncea			
India	no	−	−
B. napus			
American Purple	no	−	−
Arobidopsis thaliana			
La-0	yes	++	+++
Col-0	yes	++	+++
Ag-0	yes	++	+++
Bus-0	yes	++	+++
No-0	yes	++	+++
Raphanus sativus			
April Cross	no	−	−
Inca	no	−	−
Champion	no	−	−
Cherry Belle	no	−	−
French Breakfast	no	−	−
Icicle	no	−	−

[a]Inoculum contains TCV plus the satellite RNA D.
[b]Inoculum contains TCV plus the satellites RNA D, RNA F, and RNA C.
[c]+ = slight stunting and/or crinkled leaves, ++ = severe stunting and/or dark green crinkled leaves, +++ = severe necrosis and plant death, − = no detectable difference from mock-inoculated plants.

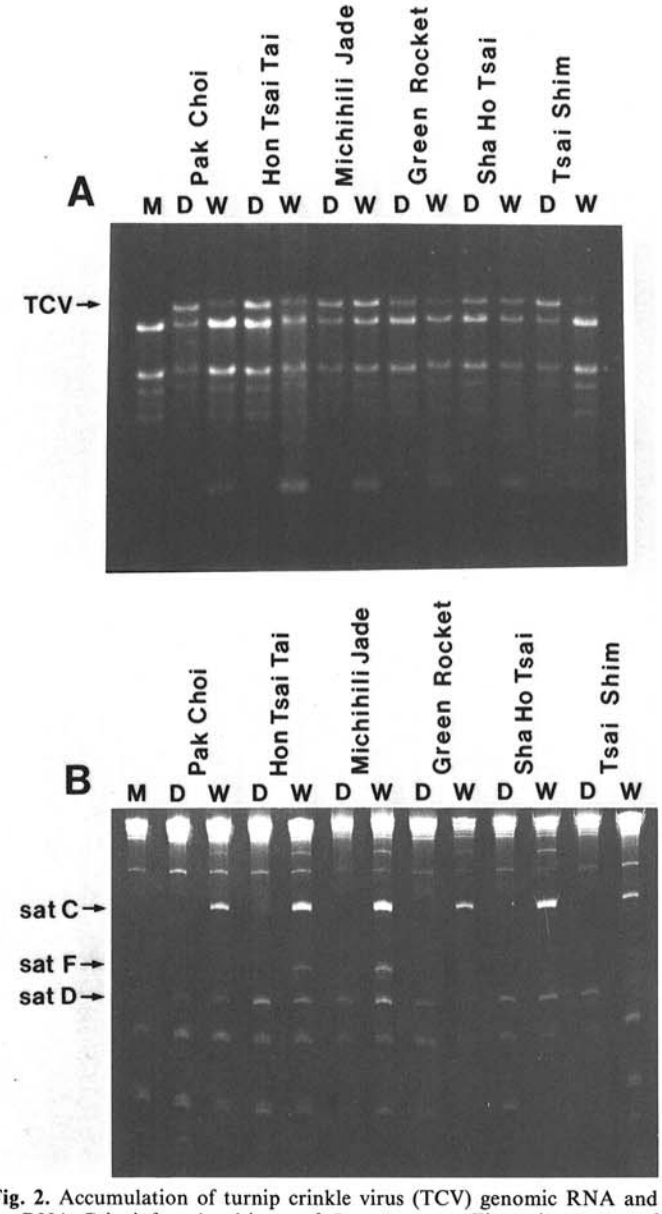

Fig. 2. Accumulation of turnip crinkle virus (TCV) genomic RNA and sat-RNA C in infected cultivars of *Brassica rapa*. Five micrograms of total RNA isolated from mock (M), TCV-m + D (D) or TCV-M (W) infected plants was subjected to electrophoresis on **A,** 1% nondenaturing agarose gels or **B,** 5% polyacrylamide/50% urea gels followed by staining with EtBr. These gels and similar ones were scanned by densitometer to estimate the relative TCV genomic and sat-RNA C levels that are presented in Table 2. Only *B. rapa* 'Pak Choi' and 'Hon Tsai Tai' exhibited disease symptoms upon infection with TCV-m + D or TCV-M.

with ethidium bromide and photographic negatives scanned by densitometer to quantitate the relative levels of genomic TCV and sat-RNA C present in each plant. The results, presented in Table 2, indicate that there was no statistical significance at the $P < 0.05$ level in the accumulation of TCV or sat-RNA C in tolerant and susceptible *B. rapa* cultivars using the Mann-Whitney U test. Therefore, we conclude that approximately equal levels of genomic TCV and sat-RNA C were present in all *B. rapa* cultivars whether or not symptoms were produced.

Because TCV-M differs from TCV-m + D by the presence of sat-RNA F as well as sat-RNA C, it is necessary to demonstrate that sat-RNA F is not also involved in symptom production. *B. rapa* and *A. thaliana* plants, which were previously found to exhibit intensified symptoms when infected with TCV-M, were inoculated with TCV-m + D along with in vitro synthesized sat-RNA C transcripts (19). There were no discernible differences in visible symptoms between plants that did or did not contain sat-RNA F (data not shown). We therefore conclude that intensified symptoms in plants infected by TCV-M versus TCV-m + D were due to the presence of sat-RNA C.

DISCUSSION

One characteristic that distinguishes plant viruses from most animal viruses is their common association with satellite RNAs. TCV is unique among plant viruses in its ability to support the simultaneous replication of three different satellite RNAs. Sat-RNA F, which differs from sat-RNA D mainly by the insertion of a 36-base sequence (19) and sat-RNA D were previously found to be avirulent on Just Right, whereas sat-RNA C was a virulent satellite, exacerbating the normally mild symptoms produced by TCV alone (1,18,20). Because cucumber mosaic virus sat-RNA can have different effects on different hosts (reviewed in 10), we were interested in determining what effects sat-RNA C might have on different hosts of TCV. For this report, we have tested 23 different cultivars from five species of crucifers for symptoms associated with TCV sat-RNA C. Our results indicate that all hosts of TCV that exhibit visible symptoms such as stunting and leaf crinkling upon viral infection produce more intense symptoms if sat-RNA C is included in the inoculum. We have also identified hosts that are symptomless when infected with TCV with or without sat-RNA C. Of the 10 cultivars of *B. rapa* tested, four were tolerant to viral infection, regardless of whether sat-RNA C was included in the inoculum. Lack of symptoms was not associated with a decrease in viral or satellite RNA accumulation.

Little is known about how plant viruses and some satellite RNAs interact with their hosts to produce disease symptoms. Recent studies with tobacco mosaic virus engineered to contain deletions in the coat protein gene indicated a possible involvement of the coat protein in symptom production (6). Because of their small size, symptom modification domains are currently being mapped for several virulent satellite RNAs. Baulcombe and co-workers (2) and Kurath and Palukaitus (12) independently determined that the determinants for yellow chlorosis and necrosis are located in distinct and separate regions of CMV sat-RNAs. Based on previous evidence, including the striking similarity in the 3′ domains of TCV and sat-RNA C, we hypothesized that the virus and satellite might produce symptoms by interaction of their 3′ sequences with a host component(s) (17,19). Further intensification of symptoms by sat-RNA C could be due to a dosage effect brought about by the presence in infected cells of approximately tenfold more satellite molecules than genomic virus (1). If this hypothesis is correct, then sat-RNA C might have no effect on host morphology if infection by TCV alone produced no symptoms. The results reported here support this hypothesis. We are currently working to identify exact sequences within the 3′ TCV-similar domain of sat-RNA C that are responsible for symptom production.

TABLE 2. Relative levels of turnip crinkle virus (TCV) genomic RNA and sat-RNA C accumulating in infected *Brassica rapa* tolerant and susceptible cultivars

B. rapa cultivar	Inoculum	Relative accumulation[a]	
		TCV	Sat-RNA C
Pak Choi	TCV-m + D	79 ± 38 (4)[b]	
	TCV-M	57 ± 22 (5)	290 ± 43 (5)
Hon Tsai Tai	TCV-m + D	92 (1)	
	TCV-M	45 ± 22 (5)	429 ± 116 (5)
Michihili Jade Pagoda	TCV-m + D	57 ± 16 (3)	
	TCV-M	75 ± 38 (3)	267 ± 160 (3)
Green Rocket	TCV-m + D	92 ± 1 (2)	
	TCV-M	68 ± 34 (2)	306 ± 88 (2)
Sha Ho Tsai	TCV-m + D	71 ± 21 (3)	
	TCV-M	49 ± 32 (4)	390 ± 247 (4)
Tsai Shim	TCV-m + D	103 ± 50 (4)	
	TCV-M	72 ± 25 (5)	263 ± 96 (6)

[a]Values represent area under peaks produced by densitometer scanning, normalized to internal rRNA controls within each lane.
[b]Numbers in parentheses are the number of plants analyzed. Standard deviations are given.

LITERATURE CITED

1. Altenbach, S. B., and Howell, S. H. 1981. Identification of a satellite RNA associated with turnip crinkle virus. Virology 112:25-33.
2. Baulcombe, D., Devic, M., Jaegle, M., and Harrison, B. 1988. Control of viral infection in transgenic plants by expression of satellite RNA of cucumber mosaic virus. In: UCLA Symposia on Molecular and Cellular Biology, New Series, Vol. 101. B. Staskowicz, P. Ahlquist, and O. Yoder, eds. Alan R. Liss, Inc., New York.
3. Broadbent, L., and Heathcote, G. D. 1958. Properties and host range of turnip crinkle, rosette and yellow mosaic viruses. Ann. Appl. Biol. 46:585-592.
4. Carrington, J. C., Keaton, L. A., Zuidema, D., Hillman, B. I., and Morris, T. J. 1989. The complete genome structure of turnip crinkle virus. Virology 170:214-218.
5. Davis, D. L., and Clarke, M. F. 1983. A satellite-like nucleic acid of arabis mosaic virus associated with hop nettlehead disease. Ann. Appl. Biol. 103:439-448.
6. Dawson, W. O., Bubrick, P., and Grantham, G. L. 1988. Modifications of the tobacco mosaic virus coat protein gene affecting replication, movement, and symptomatology. Phytopathology 78:783-789.
7. Gallitelli, D., and Hull, R. 1985. Characterization of satellite RNAs associated with tomato bushy stunt virus and five other definitive tombusviruses. J. Gen. Virol. 66:1533-1543.
8. Gerlach, W. L., Buzayan, J. M., Schneider, I. R., and Bruening, G. 1986. Satellite tobacco ringspot virus RNA: Biological activity of DNA clones and their in vitro transcripts. Virology 137:172-185.
9. Hollings, M., and Stone, O. M. 1972. Turnip crinkle virus. CMI/AAB Descriptions of Plant Viruses, No. 109.
10. Kaper, J. M., and Collmer, C. W. 1988. Modulation of viral plant diseases by secondary RNA agents. Pages 171-194 in: RNA Genetics, Vol. III. E. Domingo et al, eds. CRC Press, Boca Raton, FL.
11. Kaper, J. M., and Waterworth, H. E. 1977. Cucumber mosaic virus-associated RNA 5; Causal agent for tomato necrosis. Science 196:429-431.
12. Kurath, G., and Palukaitis, P. 1989. Satellite RNAs of cucumber mosaic virus: Recombinants constructed in vitro reveal independent functional domains for chlorosis and necrosis in tomato. Molec. Plant-Microbe Int. 2:91-96.
13. Li, X. H., Heaton, L. A., Morris, T. J., and Simon, A. E. 1989. Turnip crinkle virus defective interfering RNAs intensify viral symptoms and are generated de novo. Proc. Natl. Acad. Sci. (USA) 86:9173-9177.
14. Morris, T. J., and Carrington, J. C. 1988. Carnation mottle virus and viruses with similar properties. Pages 73-112 in: The Plant Viruses, Vol.3. R. Koenig, ed. Plenum Publishing Co., New York.
15. Murant, A. F., Rajeshwari, R., Robinson, D. J., and Raschke, J. H. 1988. A satellite RNA of groundnut rosette virus that is largely responsible for symptoms of groundnut rosette disease. J. Gen. Virol. 69:1479-1486.
16. Simon, A. E. 1988. Satellite RNAs of plant viruses. Plant Molec. Biol. Rep. 6:240-252.
17. Simon, A. E., Engel, H., Johnson, R. P., and Howell, S. H. 1988. Identification of regions affecting virulence, RNA processing and infectivity in the virulent satellite of turnip crinkle virus. EMBO J. 7:2645-2651.
18. Simon, A. E., Engel, H., and Howell, S. H. 1988. Turnip crinkle

virus satellite domains involved in virulence and processing. In: UCLA Symposia on Molecular and Cellular Biology, New Series, Vol. 101. B. Staskowicz, P. Ahlquist, and O. Yoder, eds. Alan R. Liss, Inc., New York.
19. Simon, A. E., and Howell, S. H. 1986. The virulent satellite RNA of turnip crinkle virus has a major domain homologous to the 3′ end of the helper virus genome. EMBO J. 5:3423-3428.
20. Simon, A. E., and Howell, S. H. 1987. Synthesis in vitro on infectious RNA copies of the virulent satellite of turnip crinkle virus. Virology 156:146-152.