Complementary DNA Cloning and Hybridization Analysis of Beet Western Yellows Luteovirus RNAs

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SUMMARY

Complementary DNAs to the virion RNAs of the ST9 strain of beet western yellows luteovirus (BWYV) were cloned and used for hybridization analyses. These showed that the two major virion ssRNAs, the genomic RNA, approximately 6 kb, and the 3-1 kb ST9-associated RNA, do not show detectable sequence homology. Evidence was also obtained concerning an ssRNA molecule of approximate size 0.72 kb, which hybridized with selected cDNA clones to the BWYV 6 kb genomic RNA; small amounts of it are associated with virions of ST9 and ST9-M BWYV isolates. Extracts of plants infected by ST9 and other BWYV isolates contained ssRNAs of approximately 6 kb, 2.9 kb and 0.72 kb in size. Extracts of ST9-infected plants also contained RNAs of approximately 3.1 kb and 0.48 kb which hybridized with selected cDNA clones prepared from the ST9-associated 3.1 kb virion RNA. cDNA clones of the ST9 virion 6 kb RNA also hybridized with the genomic 6 kb RNA of other BWYV isolates. None of the clones hybridized with preparations of other luteoviruses tested. No RNA molecule with a sequence related to the 3.1 kb ST9-associated virion RNA was detected in virions or plant tissues infected by other isolates of BWYV or other luteoviruses.

INTRODUCTION

Beet western yellows luteovirus (BWYV) is a very widespread and economically important plant virus. Unlike most luteoviruses, BWYV has a very wide host range, including many dicotyledonous and at least one monocotyledonous plant species (Duffus, 1960; Rochow & Duffus, 1981; Casper, 1988). The virions of BWYV and other luteoviruses are believed to be composed of a single species of ssRNA and a single capsid protein with an approximate M, of 24000 (Falk & Duffus, 1984; Waterhouse et al., 1986). However, several luteoviruses have additional RNAs in the purified virions. Some are heterologously encapsidated RNAs of related or unrelated viruses which are able to initiate infections independently from the associated luteovirus (Rochow, 1970; Falk et al., 1979; Waterhouse & Murant, 1983; Reddy et al., 1985). In at least two cases, however, the additionally encapsidated RNAs do not appear to be able to initiate independent infections (Takanami & Kubo, 1979; Falk & Duffus, 1984). Analysis of dsRNAs in extracts of luteovirus-infected plants have revealed not only genome-sized replicative form dsRNAs but also smaller dsRNAs (Gildow et al., 1983; Falk & Duffus, 1984), and recent hybridization and nucleotide sequence analysis of the genomic RNA for barley yellow dwarf luteovirus (BYDV) suggests that at least two subgenomic RNAs may be produced in BYDV-infected plants (Gerlach et al., 1987; Miller et al., 1988).

In this paper we report the results of cDNA cloning and nucleic acid hybridization experiments designed to evaluate the relationships between virion and virus-specific RNAs in ST9-infected plants and in plants infected by other BWYV and luteovirus isolates. We also show that cDNA clones to the ST9 BWYV RNAs detect infections by various BWYV isolates.
METHODS

Viruses and hosts. BWYV isolates were transferred to *Capsella bursa-pastoris* Medic. using *Myzus persicae* Sulzer, as previously described (Falk & Duffus, 1984). The ST9 isolate was maintained by aphid transmissions using only *C. bursa-pastoris* plants showing the severe symptom phenotype. The ST9-M isolate, in contrast, induces symptoms in *C. bursa-pastoris* typical of BWYV infection. It was derived by aphid-transmitting ST9 and selecting a *C. bursa-pastoris* plant that showed symptoms typical of those due to other BWYV isolates (Falk & Duffus, 1984).

The L-1 BWYV isolate was obtained from infected crisphead lettuce (*Lactuca sativa* L.) in California. BWYV isolates, R-7, G-8, G-H, G-I and G-6 are various field isolates collected from different crop and weed species and maintained in *C. bursa-pastoris*. Oat plants (*Avena sativa* L. cv. California Red) infected by the New York MAV, PAV and RPV BYDVs were maintained by aphid transfer as described (Rochow, 1969). *Physalis floridana* Rydb and *Vicia faba* plants, infected with potato leafroll luteovirus (PLRV) and subterranean clover red leaf luteovirus (SCRLV) respectively were maintained as described with healthy, uninfected plants as a control (Duffus, 1979, 1981). Virus-infected and healthy control plants were used fresh, or harvested 4 to 5 weeks after inoculation and stored at −20 °C.

Synthesis and cloning of cDNAs. BWYV virions were purified and RNAs were extracted as previously described (Falk & Duffus, 1984). In some experiments virions were stringently purified through two cycles of rate-zonal sucrose density gradient centrifugation, followed by an equilibrium density gradient centrifugation in caesium sulphate. Virion RNAs were extracted and preparations were tested for purity and homogeneity by non-denaturing electrophoresis in agarose gels.

Reverse transcription syntheses of cDNAs were done using modifications from existing protocols (Maniatis et al., 1982). Approximately 5 μg of ST9 virion RNAs were resuspended in 10 μl of sterile distilled water, denatured using 10 mM-methylmercuric hydroxide (Maniatis et al., 1982), made to 50 mM-dithiothreitol (DTT), and added to the first-strand cDNA reaction mixture containing 2 μg of random primers (pdN 6, Pharmacia), 100 μg bovine serum albumin, 500 μM each dTTP, dATP, dCTP and dGTP, 25 μCi [α-32P]dCTP (3000 Ci/mmol), 40 units of RNasin (Promega Biotec), 5 mM-DTT, 10 μl of 5 x reaction buffer (0.25 M-Tris-HCl pH 8.3, 15 mM-MgCl2, 0.375 M-KCl, 50 mM-DTT) and 500 units of Moloney murine leukaemia virus reverse transcriptase (Bethesda Research Laboratories). After 1 h at 37 °C, reactions were extracted with phenol :chloroform : isoamyl alcohol (25 : 24 : 1).

Second-strand cDNA synthesis reactions were done as described (Gubler & Hoffman, 1983). A sample of the ds cDNA was analysed on alkaline agarose gels (Maniatis et al., 1982), and the remainder was passed over Sephadex G-50 before being used for cloning.

In initial cloning experiments the double-stranded cDNAs were dC-tailed using terminal transferase (Bethesda Research Laboratories) as described by the manufacturer. The dC-tailed ds cDNAs were then annealed with dG-tailed pUC9 (Pharmacia). In later experiments ds cDNAs were blunt end-ligated to *PstI* linkers (New England Biolabs). Samples were digested with *PstI*, excess linkers were removed by chromatography through Sephadex G-50 columns and cDNAs were ligated into *PstI*-digested pUC19 using T4 DNA ligase (Maniatis et al., 1982). The resulting recombinant plasmids from both of the above protocols were cloned into competent *Escherichia coli* strain DH5α as described by the manufacturer (Bethesda Research Laboratories).

The bacteria harbouring recombinant plasmids were recovered and analysed as described (Maniatis et al., 1982). The recombinant plasmids were purified and cDNA insert sizes were estimated by digestion of recombinant plasmids using *PstI*, and electrophoresis with marker DNAs on agarose gels (Maniatis et al., 1982).

**BWYV RNA extraction and analysis.** Total RNAs were extracted from 1 g samples of *C. bursa-pastoris* plants as described (Klaassen & Falk, 1989). The 2 μL-liCl-insoluble ssRNA fractions were resuspended in sterile distilled water and examined by agarose gel electrophoresis and Northern blotting. Total RNAs and virion RNAs were denatured with glyoxal and DMSO and analysed in 1% agarose gels as described (McMaster & Carmichael, 1977; Falk & Duffus, 1984). RNAs were transferred to nitrocellulose membranes (no. BA 85, Schleicher & Schuell) by capillary blotting in 20 x SSC (SSC is 0.15 M-NaCl, 0.015 M-sodium citrate pH 7.0) (Thomas, 1980). In later experiments capillary blotting was onto Nytran membranes (Schleicher & Schuell) in 10 x SSC. Blots were baked at 80 °C for 1 to 2 h and used for hybridization or stored in sealed bags at 4 °C until use.

**Hybridization analysis.** Recombinant plasmids containing cDNA inserts of at least 400 nucleotides were labelled with [α-32P]dCTP using nick translation kits (Amersham). Blots were prehybridized and hybridized as described (Klaassen & Falk, 1989) then dried and exposed to X-Omat film (Kodak) at −70 °C in cassettes containing Lightning Plus intensifying screens.

The recombinant cDNAs were screened against each other by Southern blot analysis (Southern, 1975). *PstI* digests of recombinant plasmids were transferred by capillary alkaline blotting to Zeta-Probe membranes (Bio-Rad) and subjected to hybridization analysis with 32P-labelled recombinant plasmid probes. Results were assessed by autoradiography at −70 °C.

**Dot blots.** Samples were processed for dot blot analysis essentially as described (Meinkoth & Wahl, 1984). Leaf
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tissues (0.5 g) were processed by extracting sap using a stainless steel leaf squeezer (Piedmont Tool and Die, Six Mile, S.C., U.S.A.). Sap was collected in a microfuge tube and diluted 1:1 with 14% deionized formaldehyde and 12 × SSC. Samples were heated at 55 °C for 15 min and centrifuged at 13000 g for 4 min. Twenty-five μl aliquots were spotted onto nitrocellulose membranes in a Bio-Dot apparatus (Bio-Rad). Blots were then baked and subjected to prehybridization and hybridization as described above.

RESULTS

Analysis of cDNA clones and their hybridization reactions to BWYV RNAs

Alkaline agarose gel electrophoresis of first and second strand cDNAs showed size ranges from approximately 0.1 kb to 4 kb, with the majority of cDNAs between 0.5 kb and 1.0 kb. When these cDNAs were used for cloning, the majority of resulting recombinant plasmids contained relatively small cDNA inserts of less than 350 bp of cDNA. Forty-eight clones had cDNA inserts larger than 400 bp of cDNA.

We previously showed that when virion RNAs of the ST9 BWYV isolate are analysed by agarose gel electrophoresis, two prominent ssRNAs of approximately 6 kb and 3.1 kb are detected (Falk & Duffus, 1984). Eighteen of the above mentioned cDNA clones, nine of which were obtained by G-C tailing and nine by linker ligation, were nick-translated and used in hybridization analyses with the virion RNAs from the ST9 BWYV isolate. Eleven of the clones reacted specifically with the ST9 virion 6 kb RNA and seven reacted with the 3.1 kb RNA. None of the clones reacted with both RNAs.

Southern blot hybridization analysis was done on all clones which reacted with a given RNA (e.g. all those which reacted with the 6 kb RNA were tested with each other, as were all clones which reacted with the ST9-associated 3.1 kb RNA). The cDNAs specific for pBW1015 (650 bp), pBW1024 (430 bp), pBW1085 (590 bp), pBW2023 (650 bp), pBW2060 (650 bp) and pBW2083 (400 bp) did not contain detectable homology with each other, and all hybridized with the 6 kb genomic RNA. Similarly the cDNAs of clones pBW1052 (450 bp), pBW1066 (700 bp), pBW1074 (480 bp), pBW1088 (430 bp) and pBW2101 (550 bp) all reacted with the 3.1 kb ST9-associated RNA and did not contain detectable homology with each other by Southern blot analysis. The above recombinant clones and pBW2034 (550 bp), which also is specific for the 3.1 kb ST9-associated RNA and was related to pBW1052, pBW1066 and pBW1074 by Southern blot analysis, were used in Northern blot analyses with various BWYV RNAs.

Northern blot hybridizations were done using several of the recombinant plasmids and total RNAs extracted from BWYV-infected and uninoculated C. bursa-pastoris. These were done to identify the BWYV-specific RNAs which were produced in BWYV-infected plants and to compare these reactions with those for the BWYV virion-encapsidated RNAs. When cDNAs to the BWYV 6 kb genomic RNA were hybridized against total RNAs from ST9-, ST9 M- and L-1-infected C. bursa-pastoris, the reactions were qualitatively identical. ST9-infected C. bursa-pastoris always contained more BWYV–specific RNAs than did plants infected by other BWYV isolates. Reactions for the 6 kb genomic RNA, and RNAs of approximately 2.9 kb and 0.72 kb were seen (Fig. 1 shows the reactions for ST9 total RNAs). As shown, selected clones hybridized with only one, two or all three of these RNAs. As not all clones hybridized with all three RNAs, it is likely that the 2.9 kb and 0.72 kb RNAs contain subsets of the nucleotide sequence of the 6 kb genomic RNA. No reactions were obtained with RNA preparations from healthy C. bursa-pastoris plants.

When clones to the 3.1 kb ST9-associated RNA (pBW1066 and pBW2034) were evaluated with total RNA extracts, pBW1066 hybridized only with the 3.1 kb RNA, but pBW2034 hybridized with an additional RNA species of approximate size 0.42 kb (Fig. 1). Neither of these clones hybridized with RNA extracts from uninoculated plants or with those from plants infected with other BWYV isolates.

When these same cDNAs were used in Northern blot hybridization analyses with ST9 virion RNAs, not all of the RNAs detected in the total RNA preparations were found to be encapsidated. All cDNAs which reacted with the 6 kb genomic and 3.1 kb ST9-associated RNAs hybridized with their respective RNAs in the virion extracts (Fig. 2). However, somewhat surprisingly, strong hybridization reactions were also obtained for the 0.72 kb RNA,
Fig. 1. Hybridization reactions of total 2 M-LiCl-insoluble ssRNAs extracted from ST9 BWYV-infected C. bursa-pastoris with selected cDNA clones to the ST9 RNAs. Total ssRNAs were separated in denaturing agarose gels and blotted to Nytran membranes. Blots were probed with 32P-labelled recombinant plasmid pBW2104, pBW1085, pBW2023, pBW1066 and pBW2034 DNAs in lanes 3, 4, 5, 6 and 7, respectively. Reactions were detected by autoradiography. Lanes 1 and 2 show ethidium bromide-stained marker RNAs (Bethesda Research Laboratories RNA ladder) and total ssRNAs extracted from ST9-infected C. bursa-pastoris, respectively. Sizes (kb) of marker RNAs are given at left.

using clone pBW2023. The reaction corresponding to 0.72 kb RNA was detected for the ST9 and ST9-M isolates used here. No evidence was obtained to suggest that the subgenomic 2.9 kb RNA or the ST9-associated 0.48 kb RNA was also associated with purified virions. All the blots containing virion RNA from purified ST9 showed what we thought originally to be a reaction for the subgenomic 2.9 kb BWYV RNA. However, this seems to be an artefact. ST9 virion RNA preparations contain so much of the 3.1 kb ST9-associated RNA that a 'window' was produced on the blots at this location. All hybridizations using clones to the BWYV genomic RNA showed the very light 'window', and a dark area below it which resembled a specific hybridization signal. The 'window' was detected even when these blots were probed with pBW2104, which reacts only with the 6 kb genomic RNA and not the 2.9 kb RNA.

Hybridization reactions with other BWYV and luteovirus RNAs

Clones pBW1066 and pBW1085 were tested by Northern blot analysis for their quantitative as well as qualitative reactions with virion RNAs purified from two other BWYV isolates (L-1 and ST9-M). Neither of these showed any evidence for an RNA similar to the ST9-associated RNA when the virion RNAs were analysed by gel electrophoresis and ethidium bromide staining. Clone pBW1085 reacted with equal intensities when equivalent amounts of the ST9, L-1 and ST9-M BWYV 6 kb virion RNAs were compared. In contrast, clone pBW1066 reacted only with the ST9-associated virion 3.1 kb RNA (Fig. 3). There was no indication for even small
Fig. 2. Hybridization reactions of ST9 BWYV virion RNAs with cloned cDNAs. RNAs were extracted from stringently purified virions and separated by denaturing electrophoresis in agarose gels. RNAs were blotted to nitrocellulose membranes and hybridized with $^{32}$P-labelled recombinant plasmids to either the ST9 6 kb genomic RNA or the ST9-associated 3-1 kb RNA. Lanes 1 and 2 show ethidium bromide-stained virion RNAs of ST9 BWYV and tobacco mosaic virus, respectively. Lanes 3, 4, 5 and 6 show reactions of ST9 virion RNAs which were probed with pBW1085, pBW2023, pBW1066 and pBW2034, respectively. Upper, middle and lower arrow heads on the right indicate reactions for the 6 kb genomic RNA, ST9-associated 3-1 kb RNA and the 0.72 kb RNA, respectively. Note the 'window' visible in lanes 3 and 4 at the location corresponding to the ST9-associated 3-1 kb RNA.

Fig. 3. Northern blot hybridization analysis of virion RNAs of the L-1, ST9-M and ST9 isolates of BWYV with $^{32}$P-labelled recombinant plasmids pBW1066 and pBW1085. RNAs were extracted from purified virions and analysed by denaturing electrophoresis in agarose gels. Lanes 1 and 4 contain ST9-M RNA, lanes 2 and 5 contain L-1 RNA and lanes 3 and 6 contain ST9 virion RNAs. RNAs were blotted to nitrocellulose membranes and probed with the $^{32}$P-labelled pBW1066 in lanes 1 to 3, and pBW1085 in lanes 4 to 6. Reactions were detected by autoradiography. Arrow head on the right indicates the ST9-associated 3-1 kb RNA 'window' in lane 6.
Fig. 4. Dot blot hybridization reactions of pBW1085 and pBW1066 with sap extracts from healthy plants and plants infected by various BWYV and luteovirus isolates. Samples 1, 2, 3 and 4 are healthy plant samples of *A. sativa*, *C. bursa-pastoris*, *V. faba* and *P. floridana*, respectively. Samples 5 to 13 show BWYV isolates L-1, ST9-M, RY1R, R-7, G-8, G-H, G-1, G-6 and ST9, respectively. Sample 14 is SCRLV-infected *V. faba* and sample 15 is PLRV-infected *P. floridana*. Samples 16, 17 and 18 are *A. sativa* infected with MAV-NY, PAV-NY and RPV-NY BYDVs. The samples in (a) were probed with ³²P-labelled pBW1085 and those in (b) with ³²P-labelled pBW1066.

amounts of a similar ssRNA in the virion extracts of L-1 and ST9-M BWYV isolates. Also, evidence for the ST9-associated RNA ‘window’ can be seen in lane 6, and not in lanes 4 and 5 of Fig. 3.

Clones pBW1085 and pBW1066 were also evaluated for their abilities to detect various BWYV and other luteovirus isolates in dot blot analyses of sap extracts from various plant species. Neither clone gave detectable reactions with extracts of uninoculated plants or plants infected by other luteoviruses, including the MAV, PAV and RPV isolates of BYDV, PLRV or SCRLV. However, pBW1085 reacted with all BWYV isolates tested, including isolates from Florida, California and Idaho (Fig. 4). Signals were always very strong for greenhouse-grown plants and required radiographic exposures of less than 24 h. ST9-infected *C. bursa-pastoris* always gave a stronger signal than did *C. bursa-pastoris* plants infected by other BWYV isolates, further demonstrating that these plants contain greater amounts of BWYV genomic RNA.

The pBW1066 did not react with any other luteoviruses or BWYV isolates except for ST9. More recent experiments using a variety of plant species in greenhouse tests have shown that ST9 and common BWYV isolates can be differentially detected using pBW1085 and pBW1066, in plants of several species in addition to *C. bursa-pastoris*, including *Raphanus sativus* L., *L. sativa* L., *Thlaspi arvense* L. and *Spinacia oleracea* L., and that specific reactions for ST9 and typical BWYV isolates correlated well with aphid transmission recovery from these plants.

DISCUSSION

The data given here demonstrate that the previously detected ST9 virion ssRNAs, the 6 kb BWYV genomic RNA and 3.1 kb ST9-associated ssRNA, do not contain significant amounts of nucleotide sequence homology. There were no detectable cross-hybridizations using various cDNA clones to each of the RNAs. The detection of the 0.72 kb RNA in purified BWYV virion preparations was unexpected and we believe that this is neither an artefact nor a contaminant of the preparations because the virions were stringently purified by two cycles of rate-zonal sucrose density gradient centrifugation followed by equilibrium density gradient centrifugation in caesium sulphate.
RNAs of approximate sizes, 6 kb, 2.9 kb and 0.72 kb were detected in total RNA extracts of plants infected by ST9 and other BWYV isolates. Selected clones to the 6 kb genomic RNA reacted with one, two or all three of these RNAs demonstrating that these smaller RNAs are subsets of the nucleotide sequence of the 6 kb RNA. The size estimates for these RNAs are similar to those reported previously for replicative form BWYV dsRNAs produced in BWYV-infected plants, and those reported for the related BYDV (Falk & Duffus, 1984; Gildow et al., 1983). They also are similar in size to the three RNAs detected in BYDV-infected tissues by Northern blot analysis (Gerlach et al., 1987). Thus, it is possible that the 2.9 kb and 0.72 kb RNAs may represent subgenomic mRNAs representing various regions of the BWYV genome.

Recent nucleotide sequence analysis of the genomic RNAs of other BWYV isolates has shown that the genome organization is such that a subgenomic mRNA (approx. 2.2 kb), could be produced which would closely correspond in size to the 2.9 kb RNA detected here (Veidt et al., 1988). However, how the 0.72 kb RNA could be derived as a subgenomic mRNA from the reported genome organization for BWYV is unclear. If the 0.72 kb RNA proves to be a subgenomic mRNA, with minor amounts of it being encapsidated in BWYV virions, it may prove to be similar to the encapsidation of subgenomic mRNAs of tobacco mosaic virus and carnation mottle virus (Beachy & Zaitlin, 1977; Carrington & Morris, 1984).

Total RNA preparations from ST9-infected *C. bursa-pastoris* also showed a small 0.48 kb RNA related to the 3.1 kb ST9-associated RNA. Clone pBW2034 reacted with both these RNAs, whereas other cDNAs specific for the ST9-associated 3.1 kb RNA did not. The 0.48 kb RNA was not detected in virion preparations. A dsRNA of corresponding size has previously been detected in ST9-infected *C. bursa-pastoris* (Falk & Duffus, 1984). If this RNA represents a subgenomic mRNA of the 3.1 kb ST9-associated RNA, a protein with an *M*<sub>r</sub> of only 14000 (approximate size) could be translated from it.

None of the luteovirus or BWYV isolates tested here, except for ST9, hybridized with cDNA clones specific to the 3.1 kb ST9-associated RNA. All other BWYV isolates gave positive hybridization reactions with pBW1085, a clone specific to the 6 kb BWYV genomic RNA, whereas no positive reactions were obtained with this clone and the other luteoviruses tested here. Also, recent dot blot analyses of field-collected BWYV isolates from a variety of different crop and weed species have revealed only two out of 112 field samples tested which reacted positively with pBW1066 (data not shown). The samples were confirmed to be BWYV-infected by testing them also with pBW1085. Thus, RNAs related to the 3.1 kb ST9-associated RNA appear to be rare among field BWYV isolates.

The significance of the 3.1 kb ST9-associated RNA is presently unknown. Its presence is associated with a more severe symptom phenotype and a greater virion yield per gram of tissue for *C. bursa-pastoris* infected by ST9 compared to *C. bursa-pastoris* plants infected by other BWYV isolates (Falk & Duffus, 1984). When ST9 is aphid-transmitted to *C. bursa-pastoris*, a few plants show symptoms typical of BWYV infection and not the severe symptoms of the ST9 infections. In all cases, when we tested these plants showing typical BWYV symptoms by dot blot assays with pBW1066, they failed to give a positive reaction, but they reacted positively with pBW1085. Thus, all of the above-mentioned data suggest, but do not prove, that the ST9-associated RNA is likely to play a role in the phenotypic differences seen in *C. bursa-pastoris* plants infected by ST9 as opposed to other BWYV isolates.

The ST9-associated 3.1 kb RNA does not confer a more severe symptom phenotype on all plant species which are hosts for the ST9 isolate. For example, *R. sativus* L. cv. white icicle and *L. sativa* L. cv. Salinas are hosts for ST9 (data not shown). ST9-infected plants of these latter two species are phenotypically indistinguishable from plants infected by ST9-M or other BWYV isolates. Yet, pBW1066 reacts strongly with extracts of ST9-infected *R. sativus* and *L. sativa*, and aphid transmissions from these plants to *C. bursa-pastoris* yield the severe symptom phenotype typical of ST9.

It seems likely that the ST9-associated 3.1 kb RNA is a distinct replicating RNA (not derived from the 6 kb RNA) which is not necessary for replication of the 6 kb BWYV genomic RNA. There is no evidence that the ST9-associated 3.1 kb RNA can infect plants and replicate without the BWYV genomic RNA (Falk & Duffus, 1984), and thus biologically it is significantly
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Different from other RNAs such as those of the helper-dependent aphid-transmitted viruses which also can be encapsidated in luteovirus capsids (Falk et al., 1979; Waterhouse & Murant, 1983; Reddy et al., 1985). Considering these characteristics, the ST9-associated RNA in many ways resembles a satellite RNA. It is much larger than the satellite RNAs, of approximately 350 nucleotides of tobacco ringspot virus and cucumber mosaic virus, and may be more like the satellite RNAs reported in associations with some nepoviruses (Francki, 1985; Pinck et al., 1988).


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